Biologic Oxidation

1

Krebs Cycle

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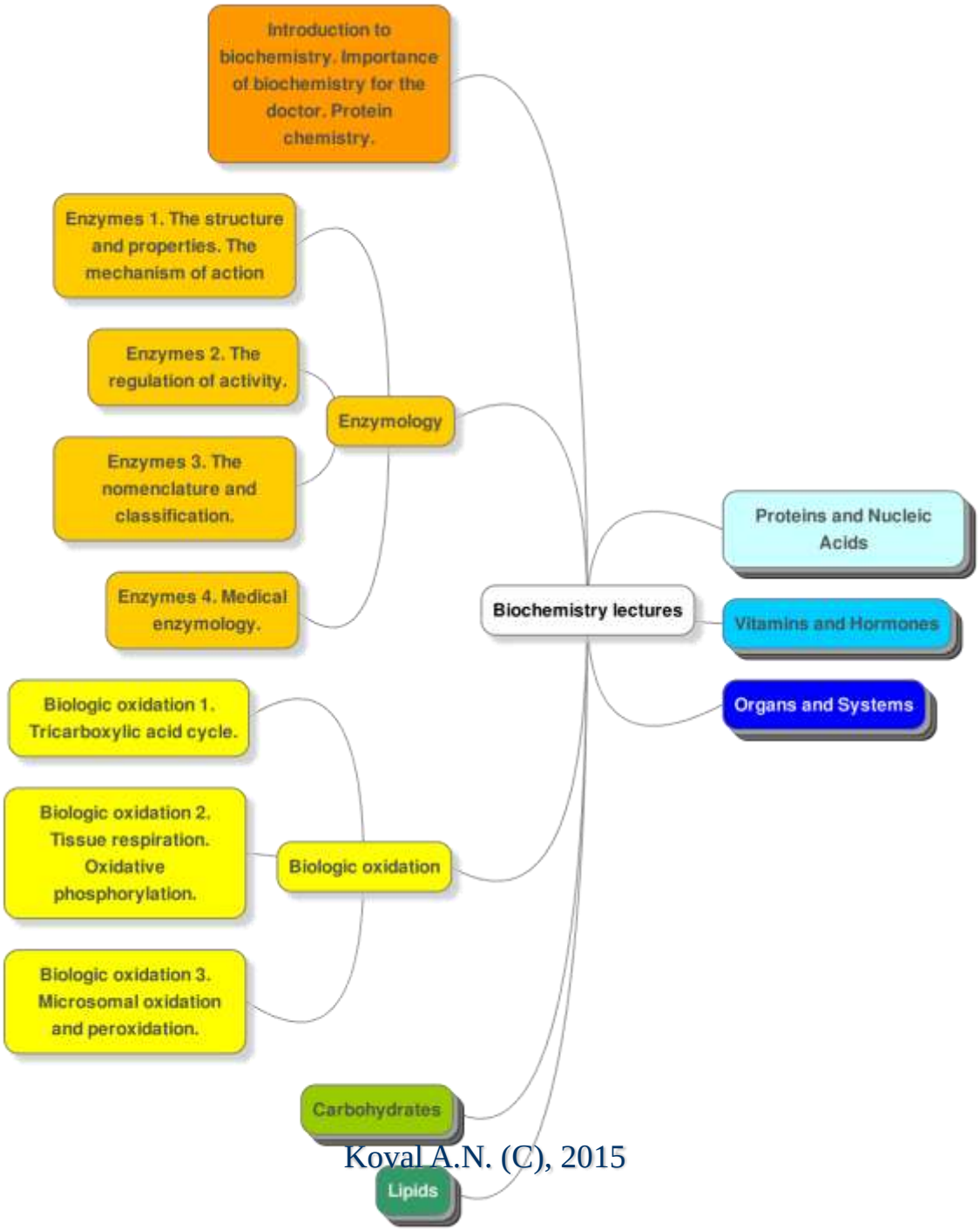
PhD, senior lecturer


Introduction to biochemistry. Importance of biochemistry for the doctor. Protein chemistry.
Enzymes 1. The structure and properties. The mechanism of action
Enzymes 2. The regulation of activity.
Enzymology
Enzymes 3. The nomenclature and classification.
Enzymes 4. Medical enzymology.
Biologic oxidation 1. Tricarboxylic acid cycle.
Biologic oxidation 2. Tissue respiration. Oxidative phosphorylation.
Biologic oxidation
Biologic oxidation 3. Microsomal oxidation and peroxidation.
Carbohydrates
Lipids
Biochemistry lectures
Proteins and Nucleic Acids
Vitamins and Hormones
Organs and Systems

Content

- Bioenergetics. Historical Background
- Biologic Oxidation
- Citric Acid Cycle (Krebs Cycle)

Bioenergetics

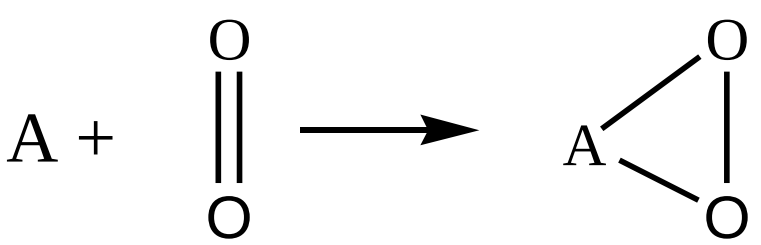
- **Bioenergetics** is the study of the energy changes accompanying biochemical reactions.

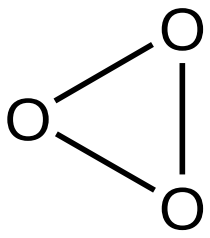
Historical Background

- Ancient conceptions. 4 elements. **Aristotle** vs **Plato** about the role of air.
- Early 1700's. **Georg Ernst Stahl** developed the **phlogiston theory** *(revise 1-st lecture)*.
- 1770's. **Carl Scheele** and **Joseph Priestley** discovered **oxygen**.
- Late 1700's. **Antoine Lavoisier** stated the law of the conservation of mass and proposed the oxygen theory of combustion.
- A. Lavoisier found similarities of burning and respiration by products.

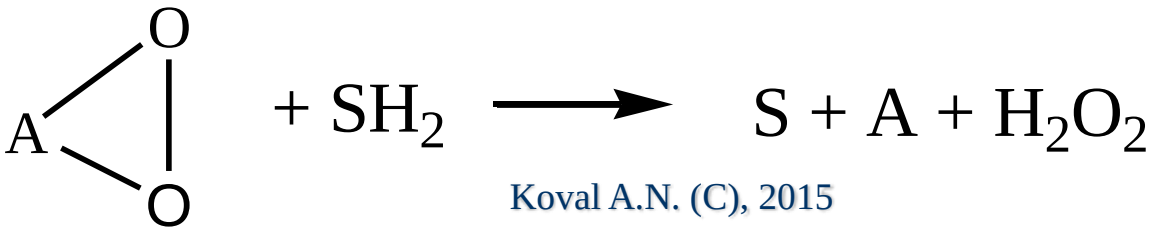
Oxygen Activation Theory

- XIX c. – A. Bakh and Engler. Peroxidation or oxygen activation theory.
  - 1840: Shönbein discovered **ozone**. O_3. More reactive oxygen.
  - Bakh developed this fact into an idea:





Hypothetical structure of ozone.



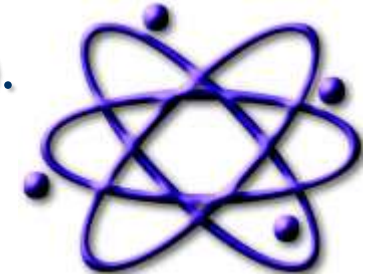
Critical Thinking about Bakh's Theory

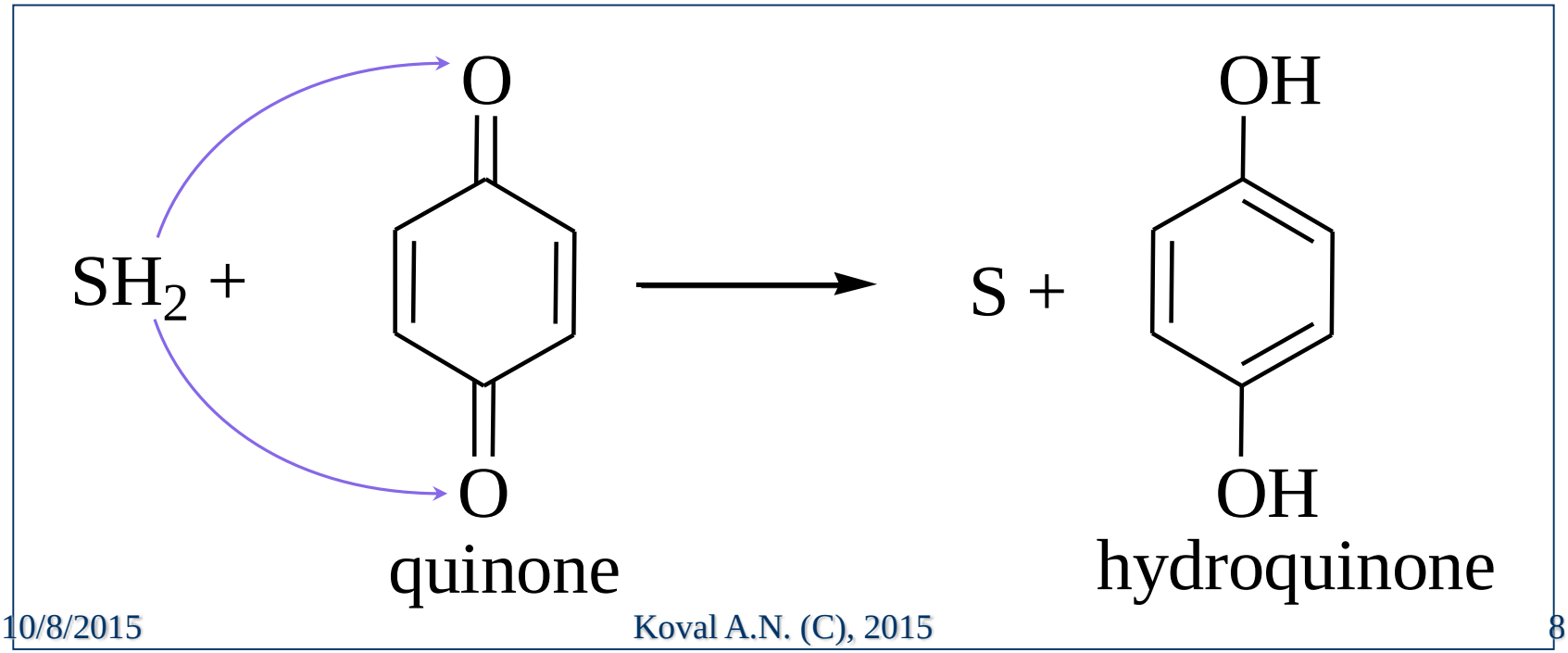
1. High activity of oxygenases was not found in the living organisms.
2. High H_2O_2 concentration was not found in the living organisms.
3. The enzymes for H_2O_2 degradation were found in the living organisms (catalase and peroxydases).

$H_2O_2 \rightarrow H_2O + O$	(*by catalase*)
$H_2O_2 + 2G\text{-}SH \rightarrow 2H_2O + G\text{-}S\text{-}S\text{-}G$	(*by glutathione peroxydase*)

Palladin-Wieland Theory

- 1911 – Ernest Rutherford presented his theory of atomic structure.
- 1913 – N. Bohr, atomic theory (nucleus, electrons).
  - Other understanding of redox processes.
- 1912 – A. Palladin and H. Wieland theory.
  1. Anaerobic stage: $SH_2 + R = S + RH_2$ (see fig.)
  2. Aerobic stage: $RH_2 + 1/2\ O_2 \rightarrow R + H_2O$.

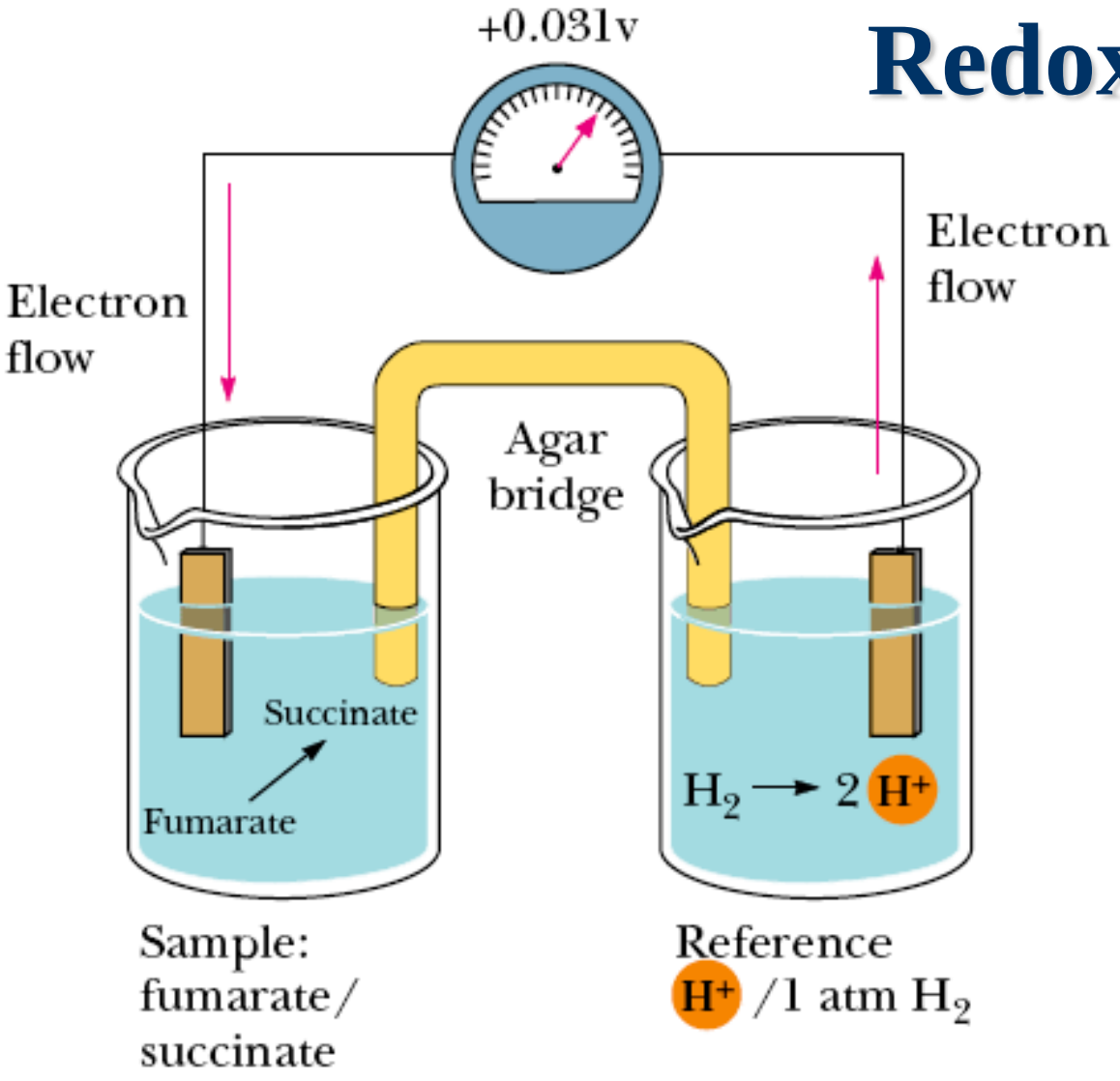




Chromogenes and Hystohematines

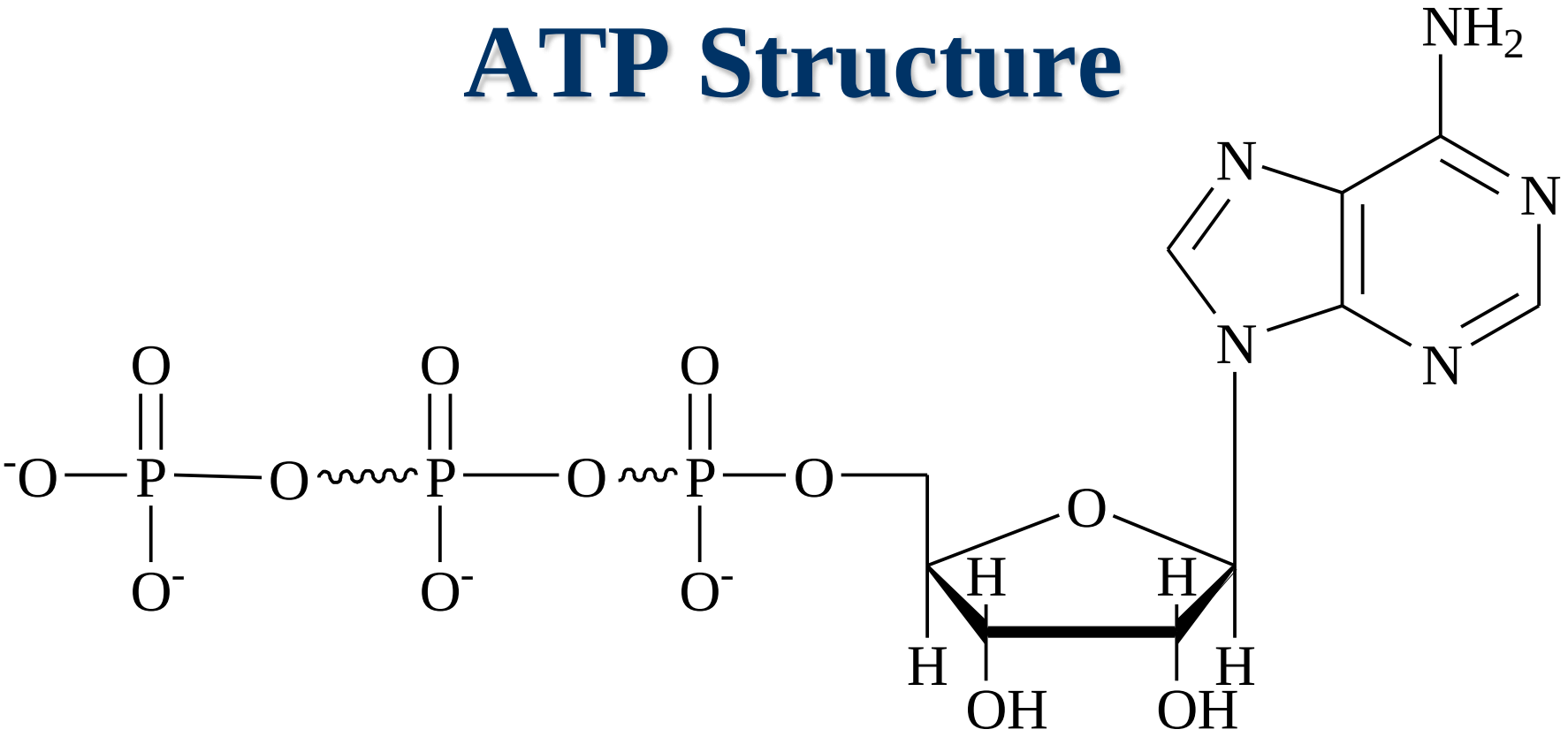
- These intermediate electron acceptors were named chromogenes, as they gain their color in oxidized form.
  - As chromogenes can serve FMN, FAD, NAD^+, $NADP^+$.
- 1925 – **hystohematines** were found (cytochromes)
- 1932 – acad. Engelgardt: oxidation coupled with phosphorylation (ADP + P_i → ATP).

Redox Reactions. Redox Potential



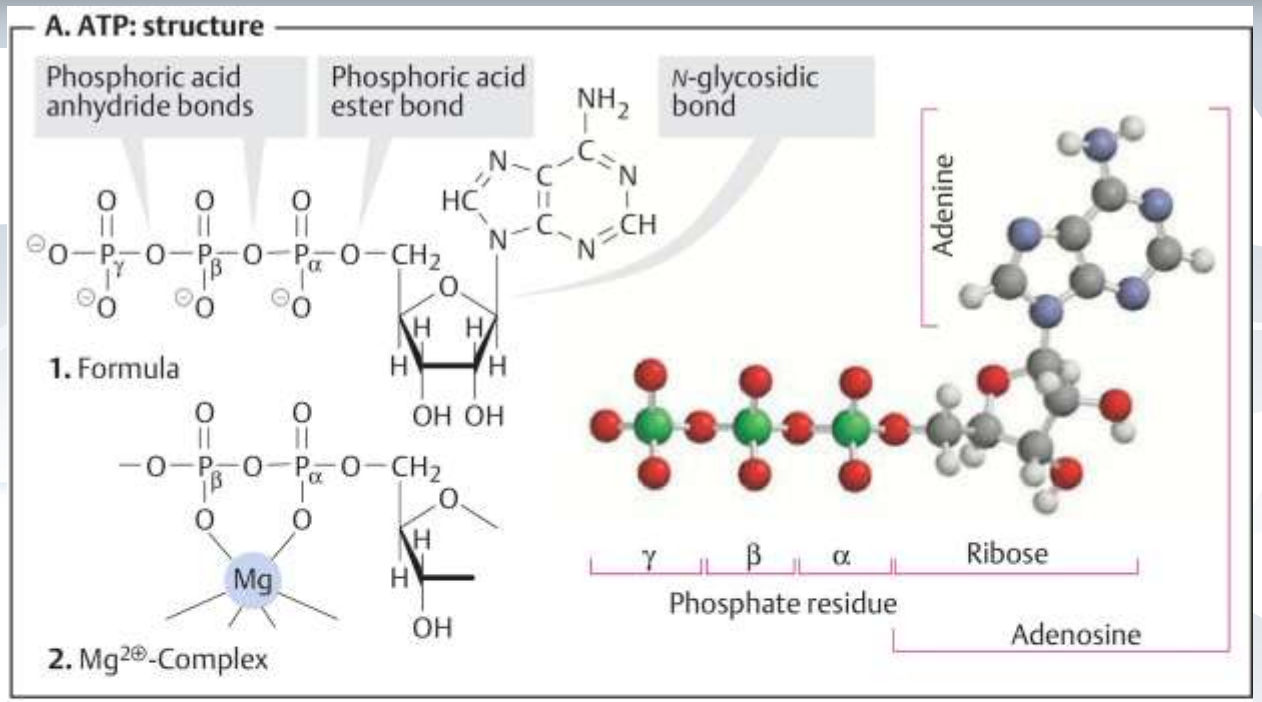
- Reactions of the transfer of electrons from the donor to acceptor.
- Redox potential is the observed voltage.
- The redox potential of H^+:H_2 couple is defined as zero.

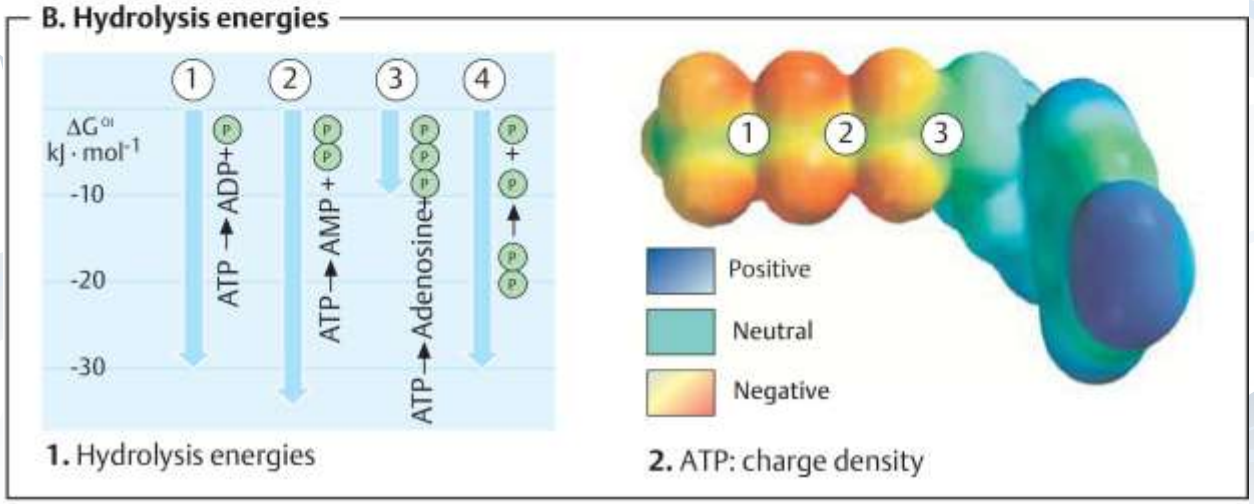
ATP Structure



- ATP is the principal high-energy compound in all living cells.
- It contains 2 macroergic bonds.

ATP: Energy





- In ATP, the oxygen atoms of all 3 phosphate residues have similarly strong negative charges (orange), while the phosphorus atoms represent centers of positive charge.
- One of the reasons for the instability of phosphoric anhydride bonds is the repulsion between these negatively charged oxygen atoms, which is partly relieved by cleavage of a phosphate residue.

Macroergicity of ATP

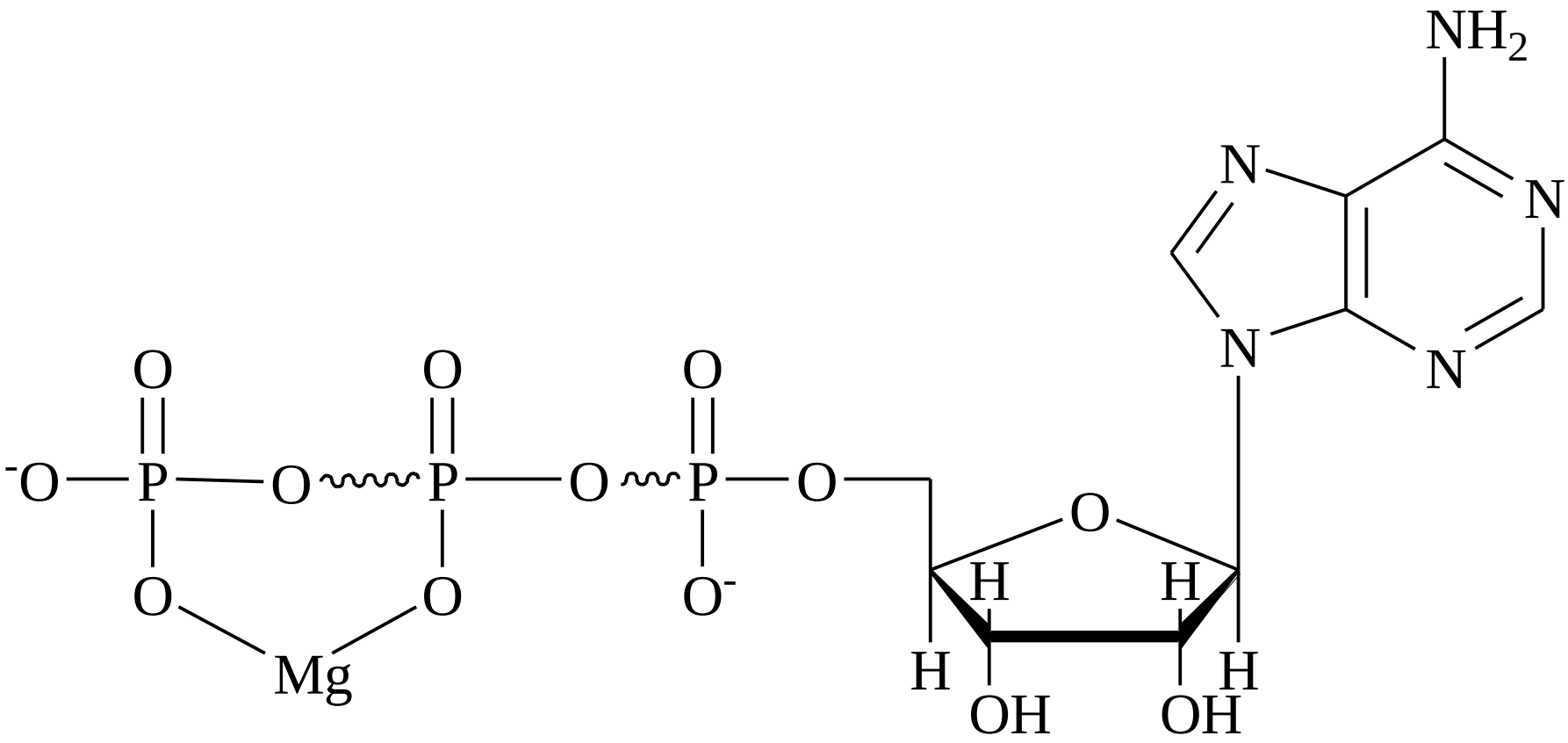
1. Negative phosphate “tail”. Strong repulsing.
2. $ATP^{4-} \rightarrow ADP^{3-} + Pi^{2-} + H^{+}$
    1. $[ATP^{4-}] = [ADP^{3-}] = [Pi^{2-}] = [H^{+}] = 10^{-3}$ M
    2. If $[H^{+}]$ were 10^{-3} M, pH=3. But pH=7, so $[H^{+}] = 10^{-7}$, and the chemical equilibrium is shifted right.
3. Resuming:

    $G(ATP^{4-}) >> G(ADP^{3-}) + G(Pi^{2-}) + G(H^{+})$

Standard Free Energy of Hydrolysis of Some Organophosphates of Biochemical Importance

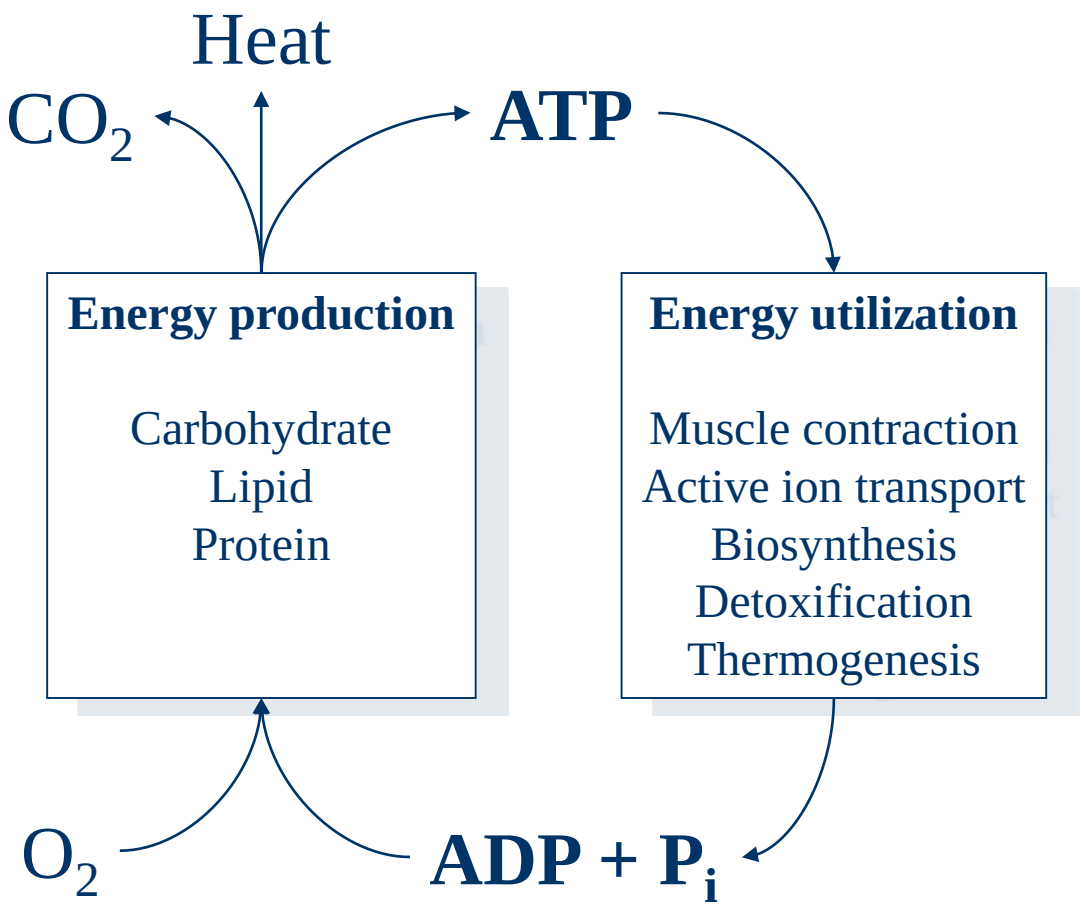
Compound	G^0	
	kJ/mol	kcal/mol
Phosphoenolpyruvate	–61.9	–14.8
Carbamoyl phosphate	–51.4	–12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	–49.3	–11.8
Creatine phosphate	–43.1	–10.3
$ATP \rightarrow AMP + PP_i$	–32.2	–7.7
$ATP \rightarrow ADP + P_i$	**–30.5**	**–7.3**
Glucose 1-phosphate	–20.9	–5.0
PP_i	–19.2	–4.6
Fructose 6-phosphate	–15.9	–3.8
Glucose 6-phosphate	–13.8	–3.3
Glycerol 3-phosphate	–9.2	–2.2

Mg^{2+} stabilizes the structure of ATP



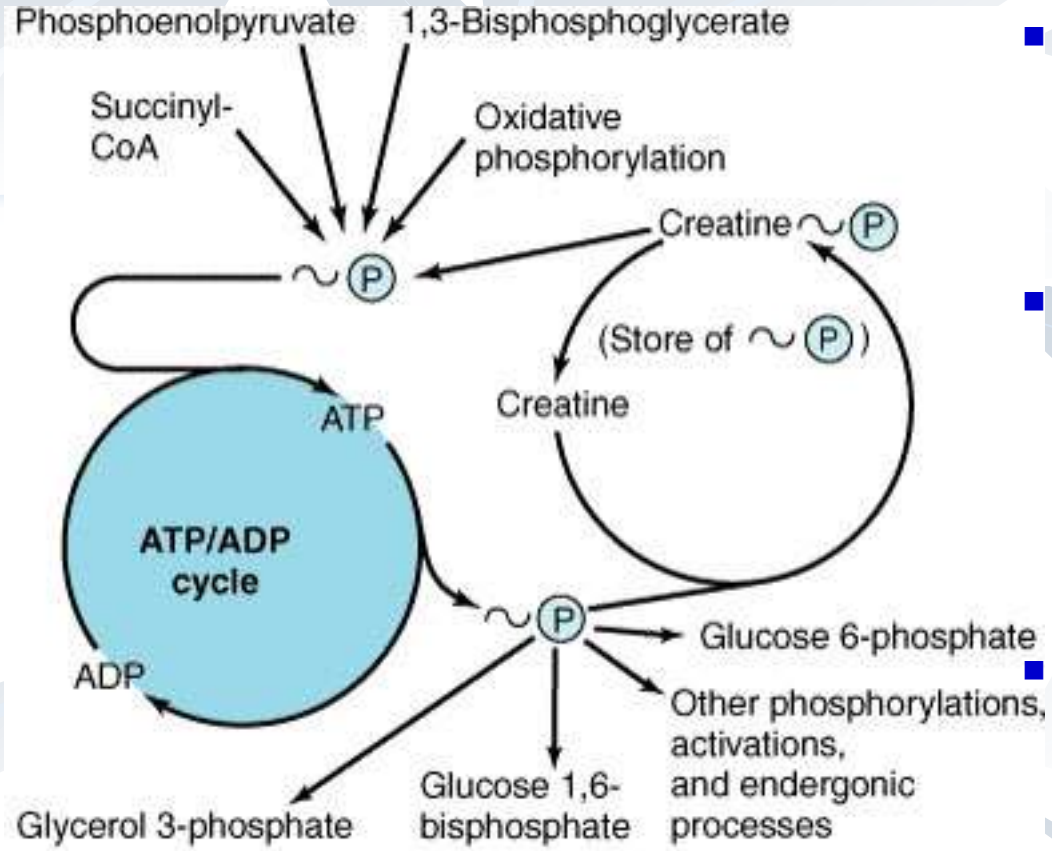
- … otherwise the ATP molecule is unstable.

ATP-ADP cycle



The principle of **ATP-ADP cycle** is that fuel oxidation generates ATP, and hydrolysis of ATP to ADP provides the energy for most of the work in the cell. ATP is the cells "energy currency". ATP supply should be constantly replenished through the use of O_2 for fuel oxidation.

High-Energy Phosphates Act as the "Energy Currency" of the Cell



- **ATP** - a donor of **high-energy phosphate**.
  - ADP can accept high-energy phosphate to form ATP from more energy-rich compounds.
- An **ATP/ADP cycle** connects those processes that
  - **generate ~P** to those processes that
  - **utilize ~P**, continuously consuming and regenerating ATP.
- This occurs at a very rapid rate, since
  - the total ATP/ADP pool is extremely small
  - sufficient for only a few seconds.

High-Energy Phosphates and ATP/ADP-cycle

High-Energy Phosphates

Compound	G^0	
	kJ/mol	kcal/mol
Phosphoenolpyruvate	–61.9	–14.8
Carbamoyl phosphate	–51.4	–12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	–49.3	–11.8
Creatine phosphate	–43.1	–10.3
ATP → AMP + PP_i	–32.2	–7.7
ATP → ADP + P_i	**–30.5**	**–7.3**
Glucose 1-phosphate	–20.9	–5.0
PP_i	–19.2	–4.6
Fructose 6-phosphate	–15.9	–3.8
Glucose 6-phosphate	–13.8	–3.3
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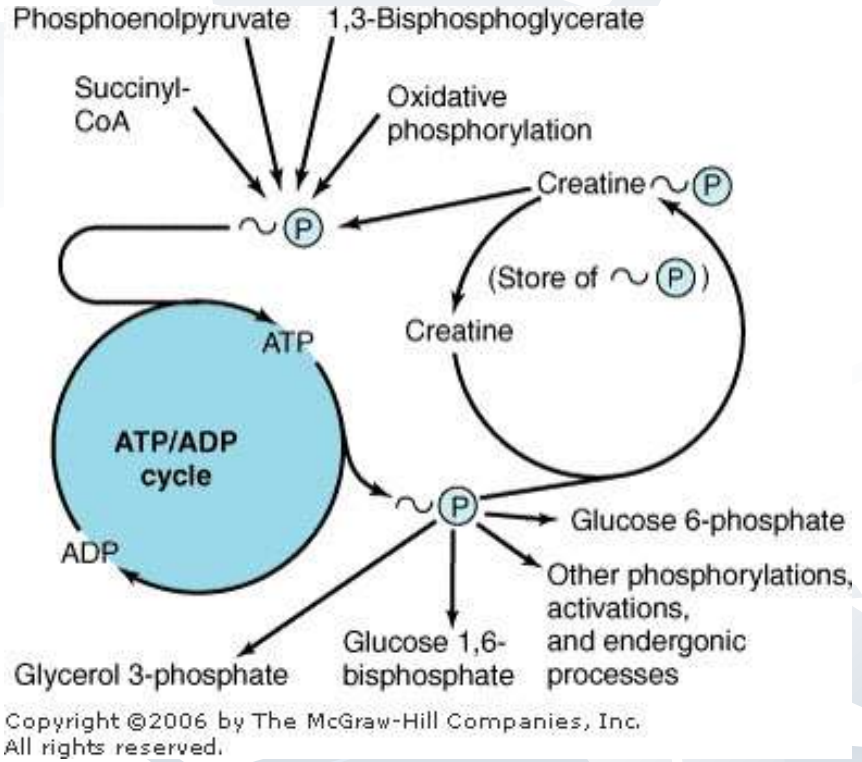
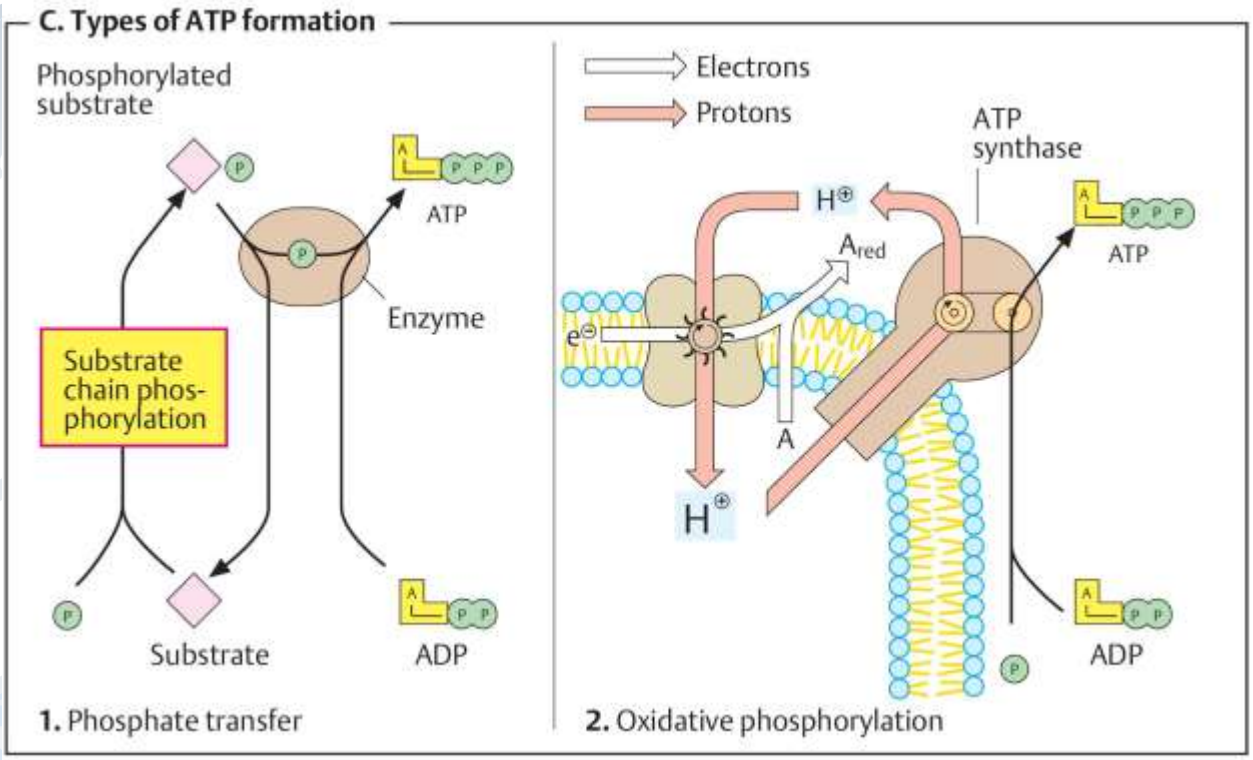
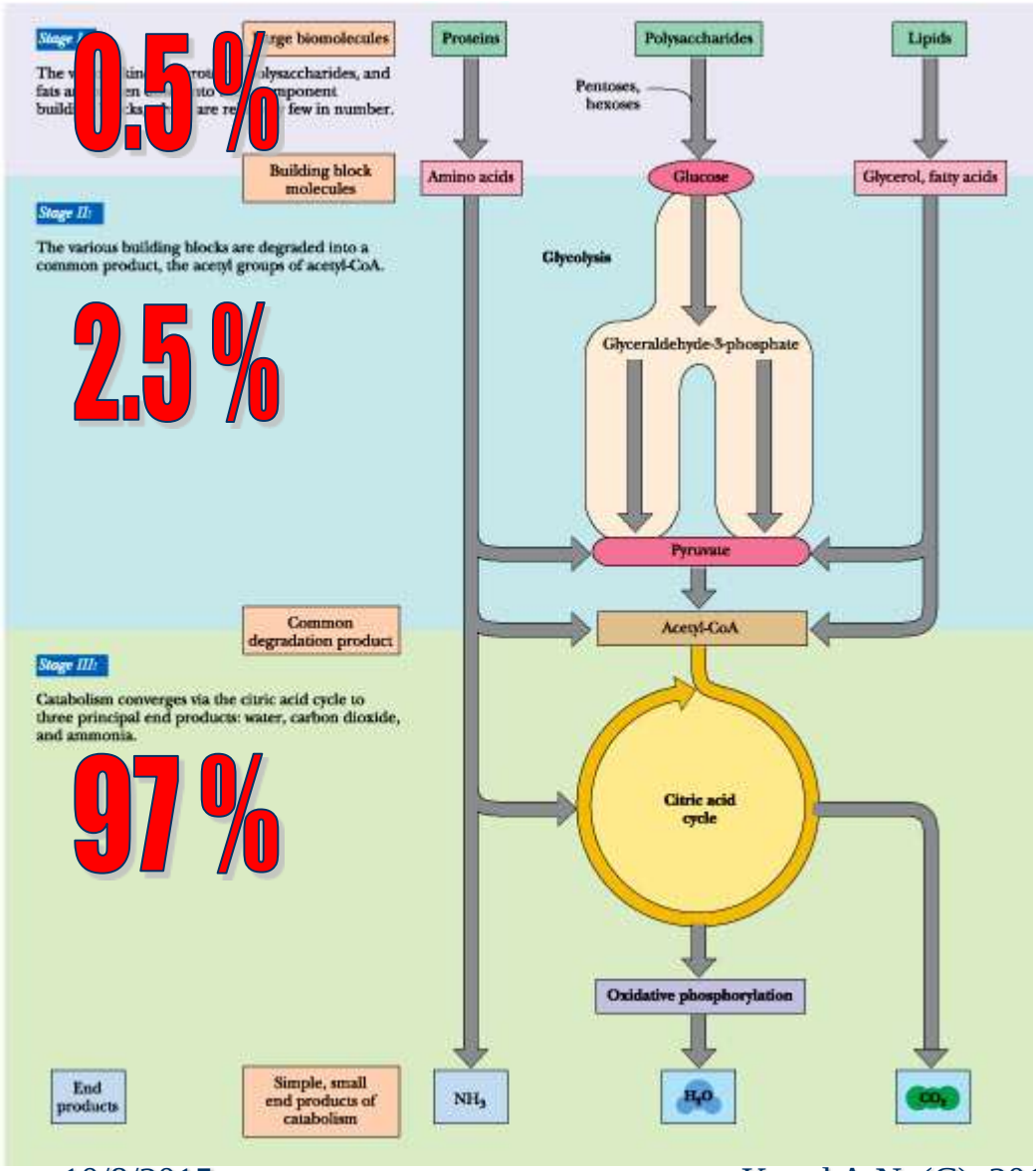


Types of ATP Formation



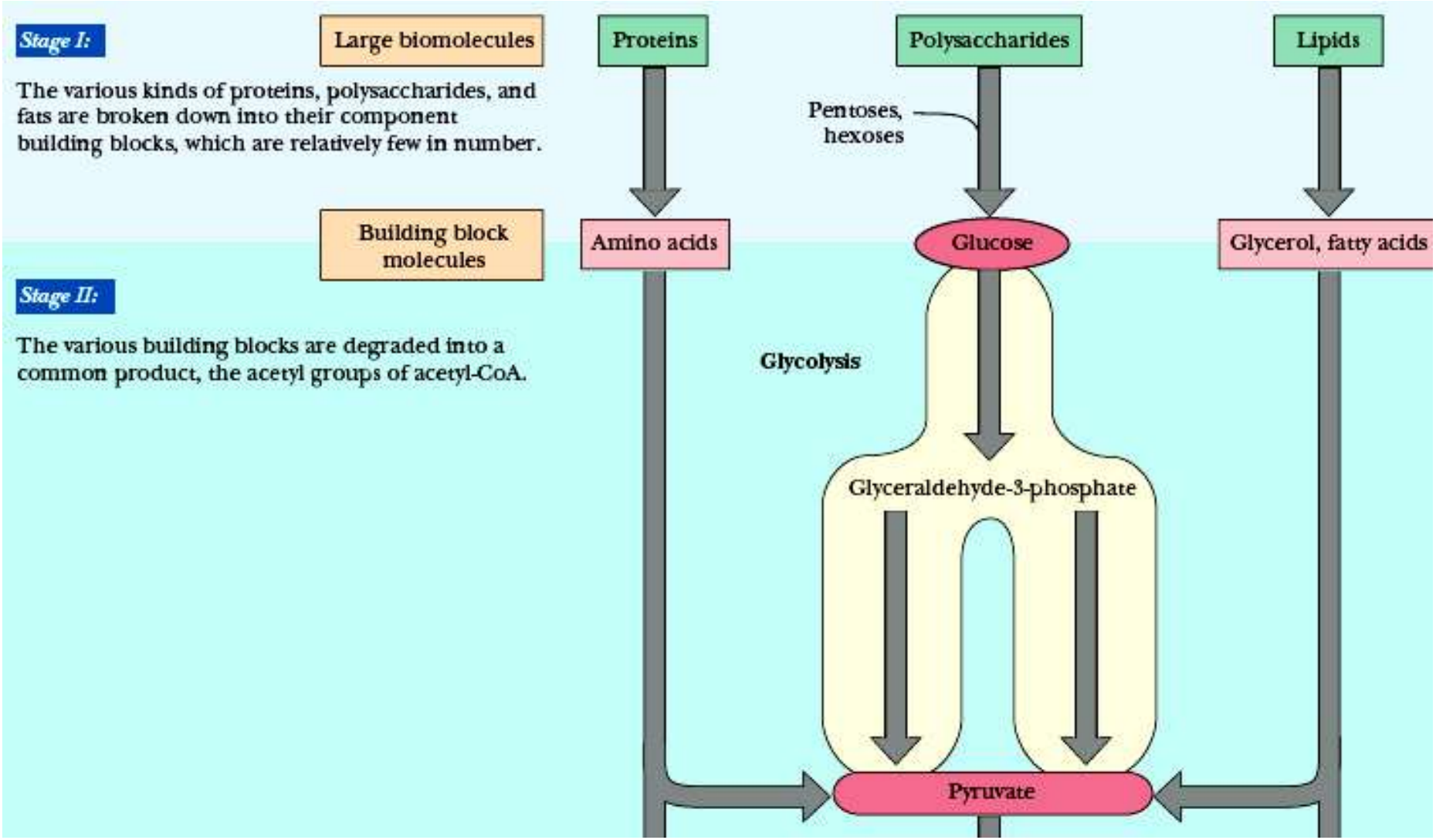
- Processes that raise inorganic phosphate to this type of high potential are called **substrate level phosphorylations.**
- **Oxidative phosphorylation** takes place in mitochondria (or in chloroplasts) and is energetically coupled to a proton gradient over a membrane.

Formation of Substrates for BO

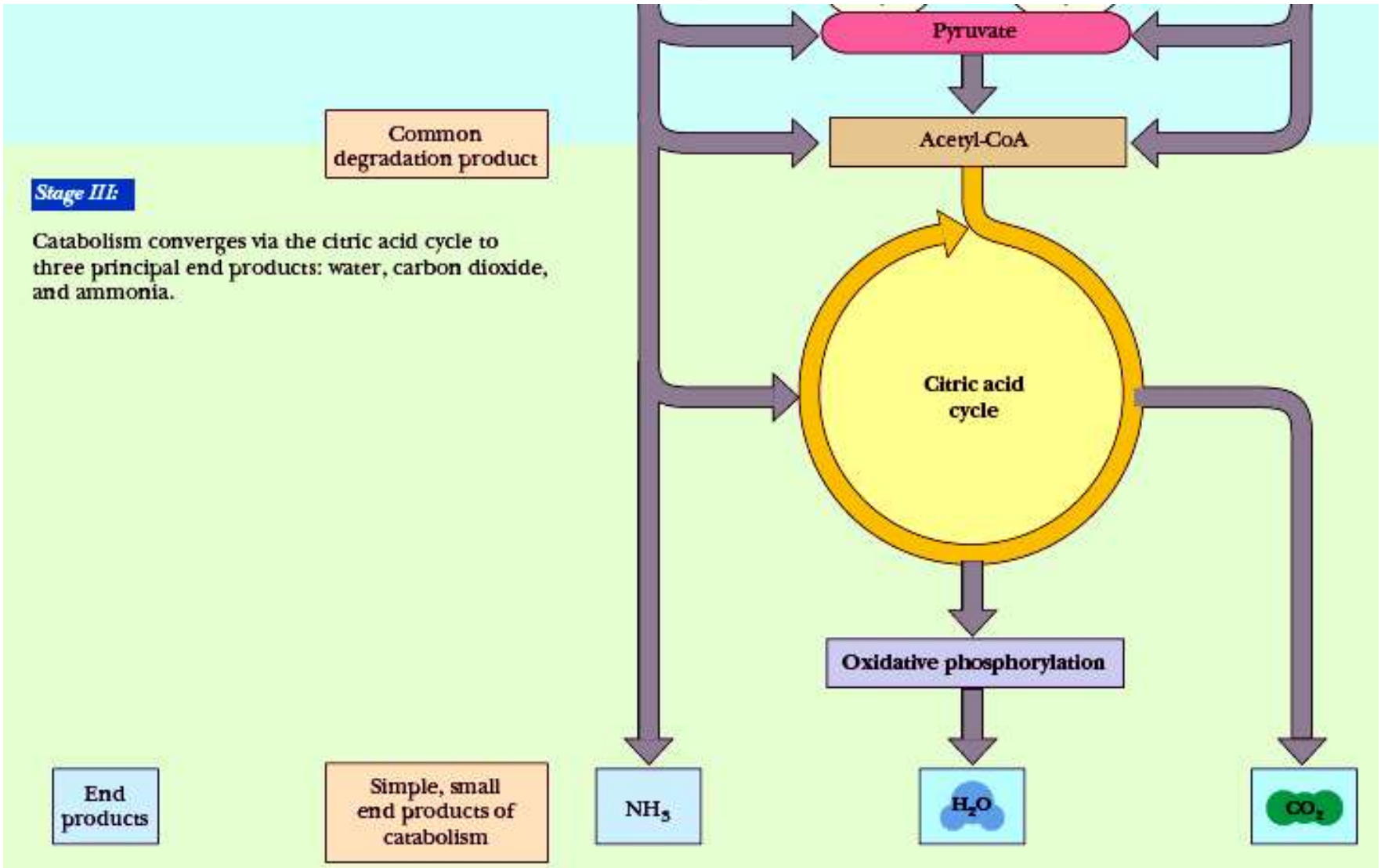


- **Stage I:** Proteins, polysaccharides, and lipids are broken down into their component building blocks, which are relatively few in number.
- **Stage II:** The various building blocks are degraded into the common product, the acetyl groups of acetyl-CoA.
- **Stage III:** Catabolism converges to three principal end products: water, carbon dioxide, and ammonia.

Stages 1 and 2



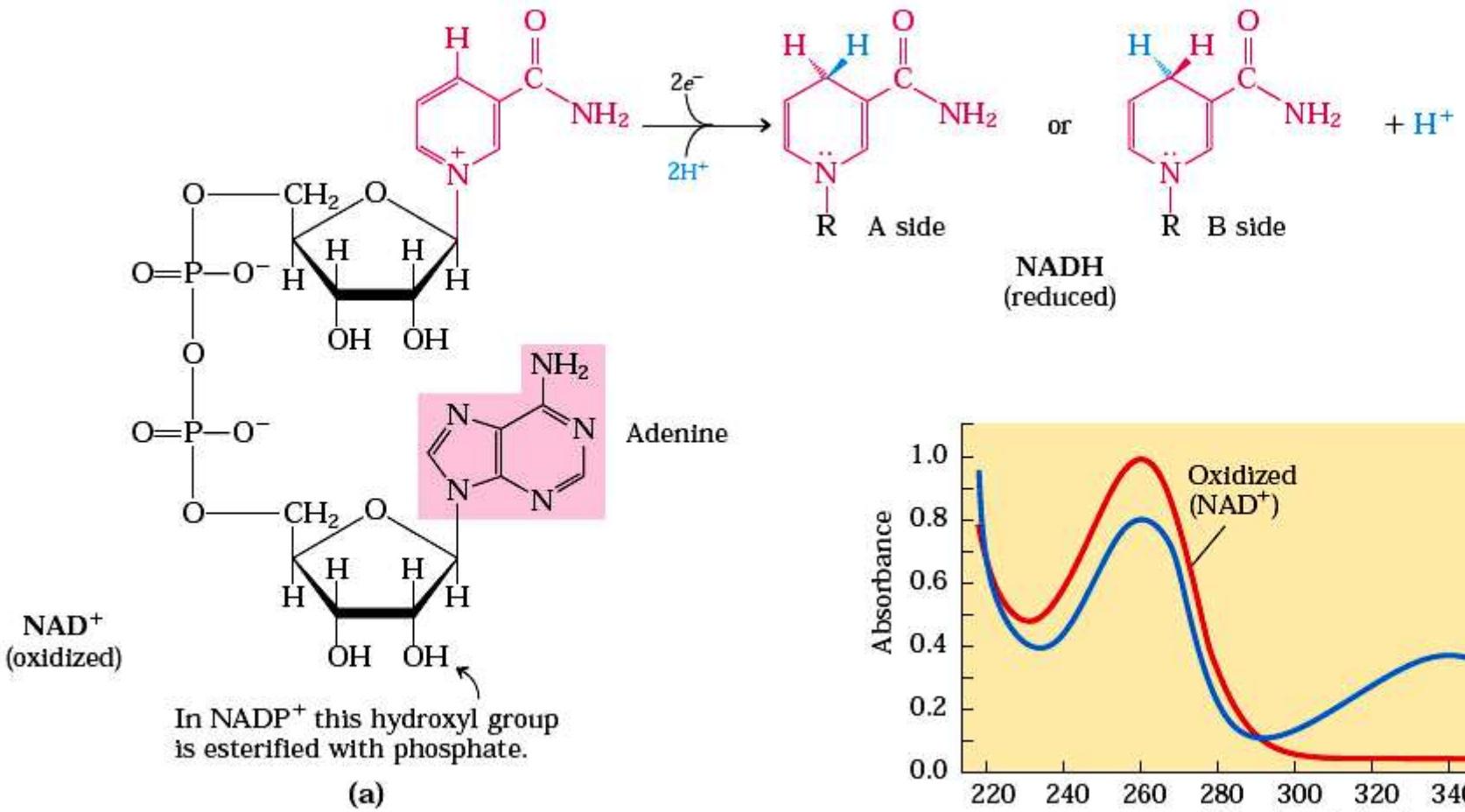
Stage 3


Pyruvate
Common degradation product
Acetyl-CoA
Stage III:
Catabolism converges via the citric acid cycle to three principal end products: water, carbon dioxide, and ammonia.
Citric acid cycle
Oxidative phosphorylation
End products
Simple, small end products of catabolism
NH_3
H_2O
CO_2

Enzymes and Coenzymes of Biologic Oxidation

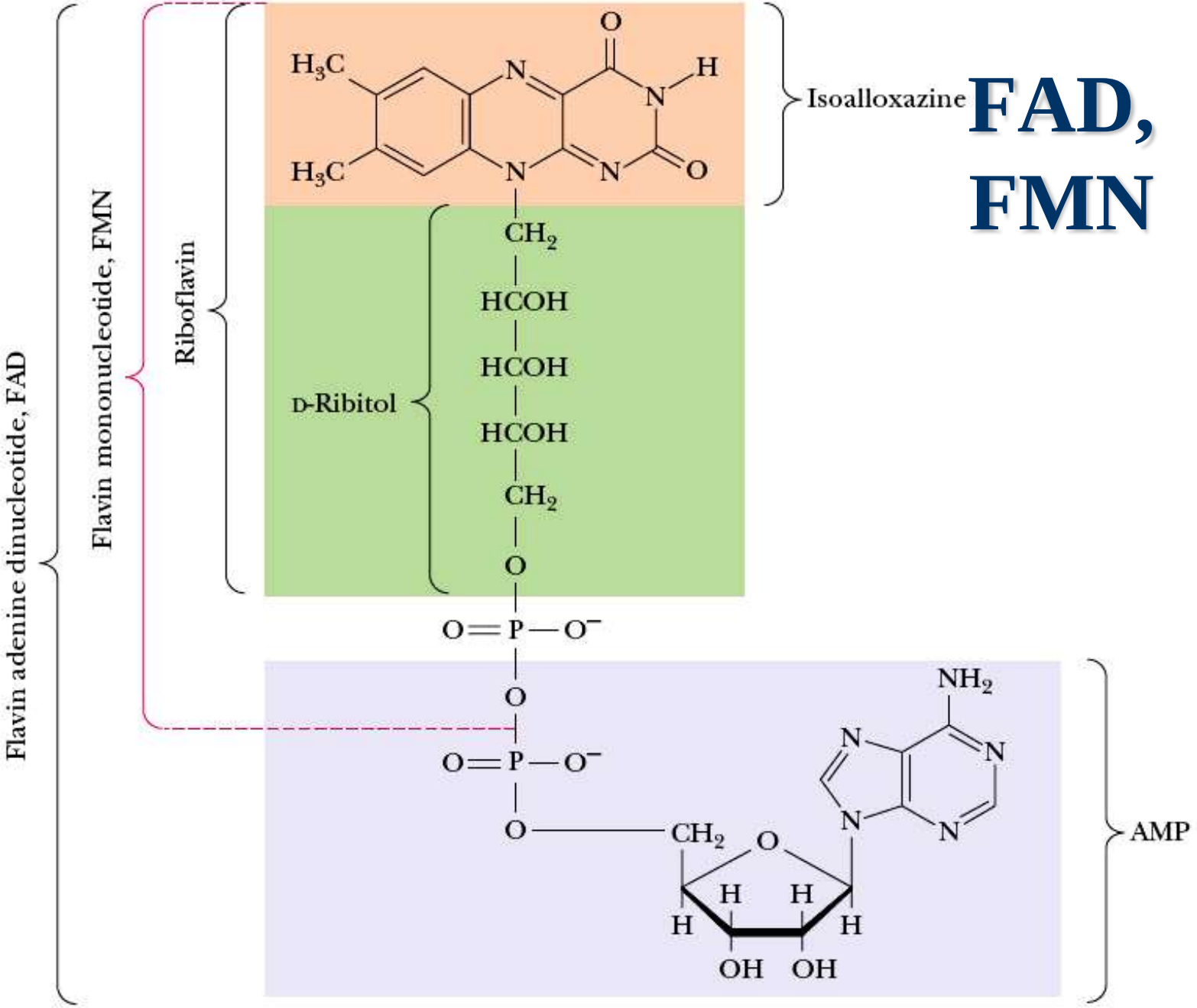
- Oxidoreductases
  - Oxidases
  - Dehydrogenases
    - Pyridine (NAD^+, $NADH^+$)
    - Flavine (FMN, FAD)
  - Hydroperoxidases
  - Oxygenases.

NAD(P)$^+$

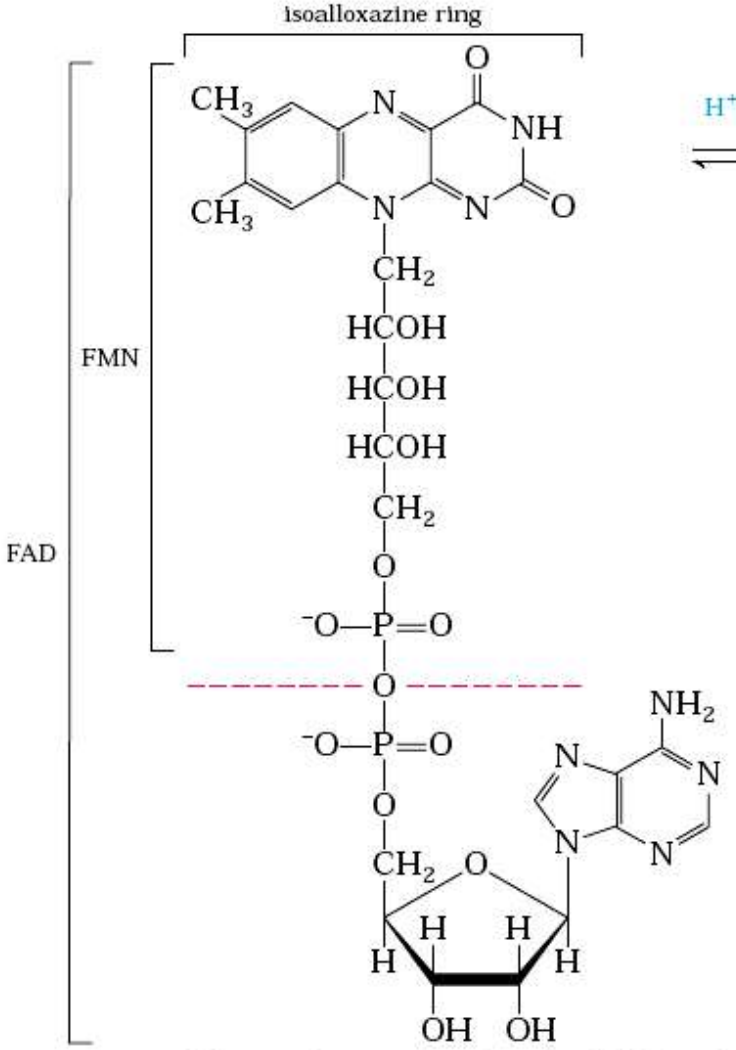

2e⁻
2H⁺
or
+ H⁺
A side
B side
NADH
(reduced)
Adenine
NAD⁺
(oxidized)
In NADP⁺ this hydroxyl group
is esterified with phosphate.
(a)

Oxidized
(NAD⁺)
Reduced
(NADH)
Absorbance
1.0
0.8
0.6
0.4
0.2
0.0
220
240
260
280
300
320
340
360
380
Wavelength (nm)
(b)

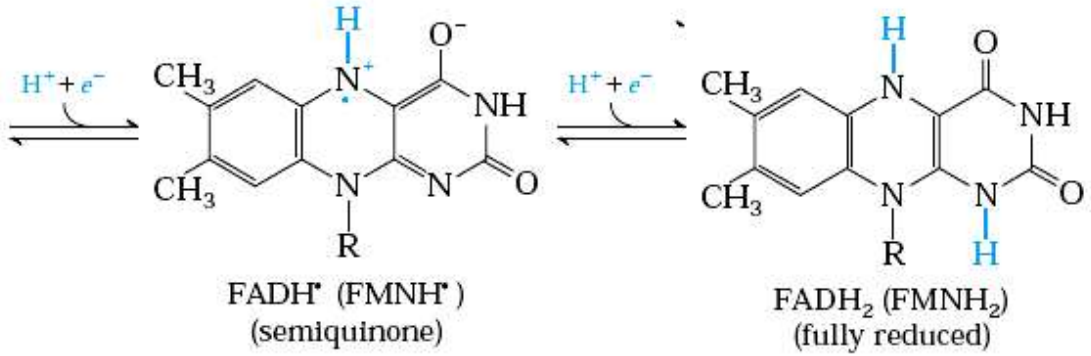
FAD, FMN


Flavin adenine dinucleotide, FAD
Flavin mononucleotide, FMN
Riboflavin
Isoalloxazine
D-Ribitol
AMP

Structures of FAD and FMN



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)



FADH• (FMNH•)
(semiquinone)

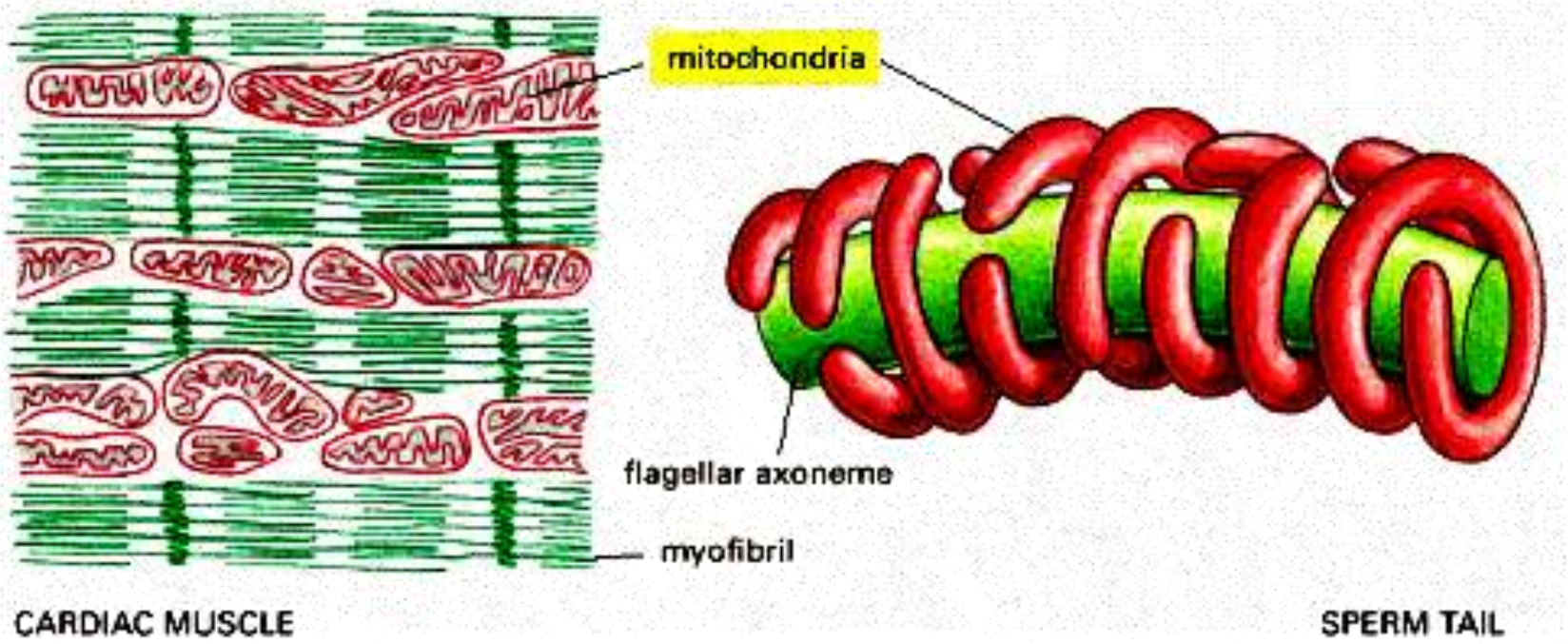
$FADH_2$ ($FMNH_2$)
(fully reduced)

FMN consists of the structure above the dashed line on the FAD (oxidized form).

The flavin nucleotides accept two hydrogen atoms (two electrons and two protons), both of which appear in the flavin ring system.

When FAD or FMN accepts only one hydrogen atom, the semiquinone, a stable free radical, forms.

Mitochondria: Localization



- **Mitochondria**: elongated cylinders, ∅ 0.5 - 1.0 μm.
  - Time-lapse microcinematography of living cells shows that mitochondria are mobile and plastic organelles, constantly changing their shape and even fusing with one another and then separating again.

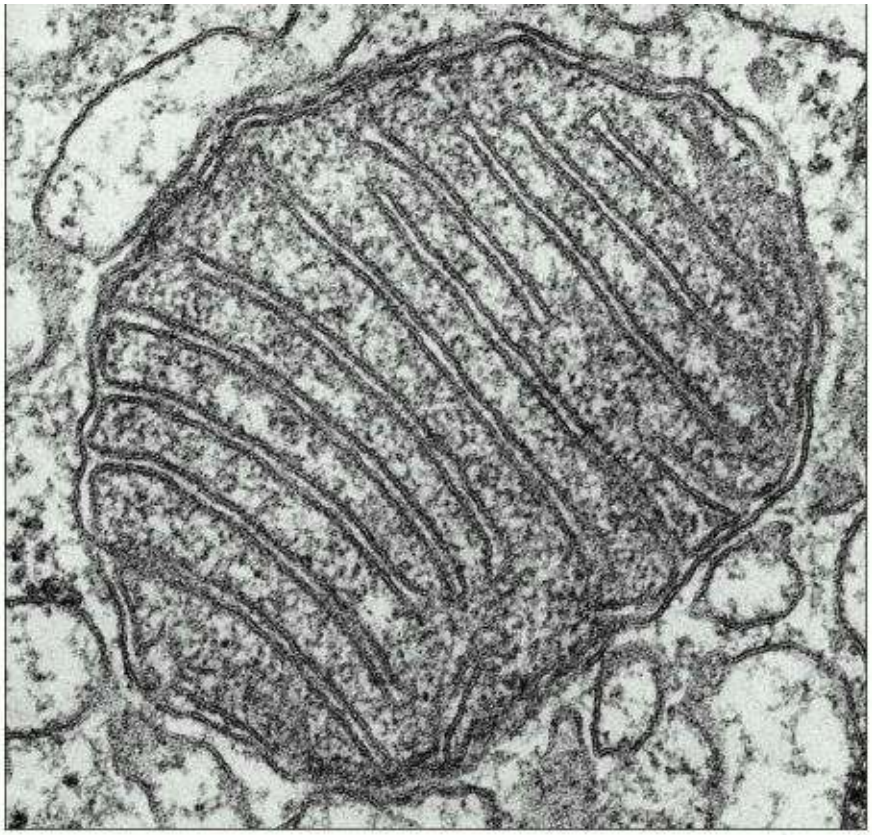
Mitochondria: Localization (cont'd)

- The mitochondria in some cells form long moving filaments or chains.
- In others they remain fixed in one position where they provide ATP directly.

Mitochondria

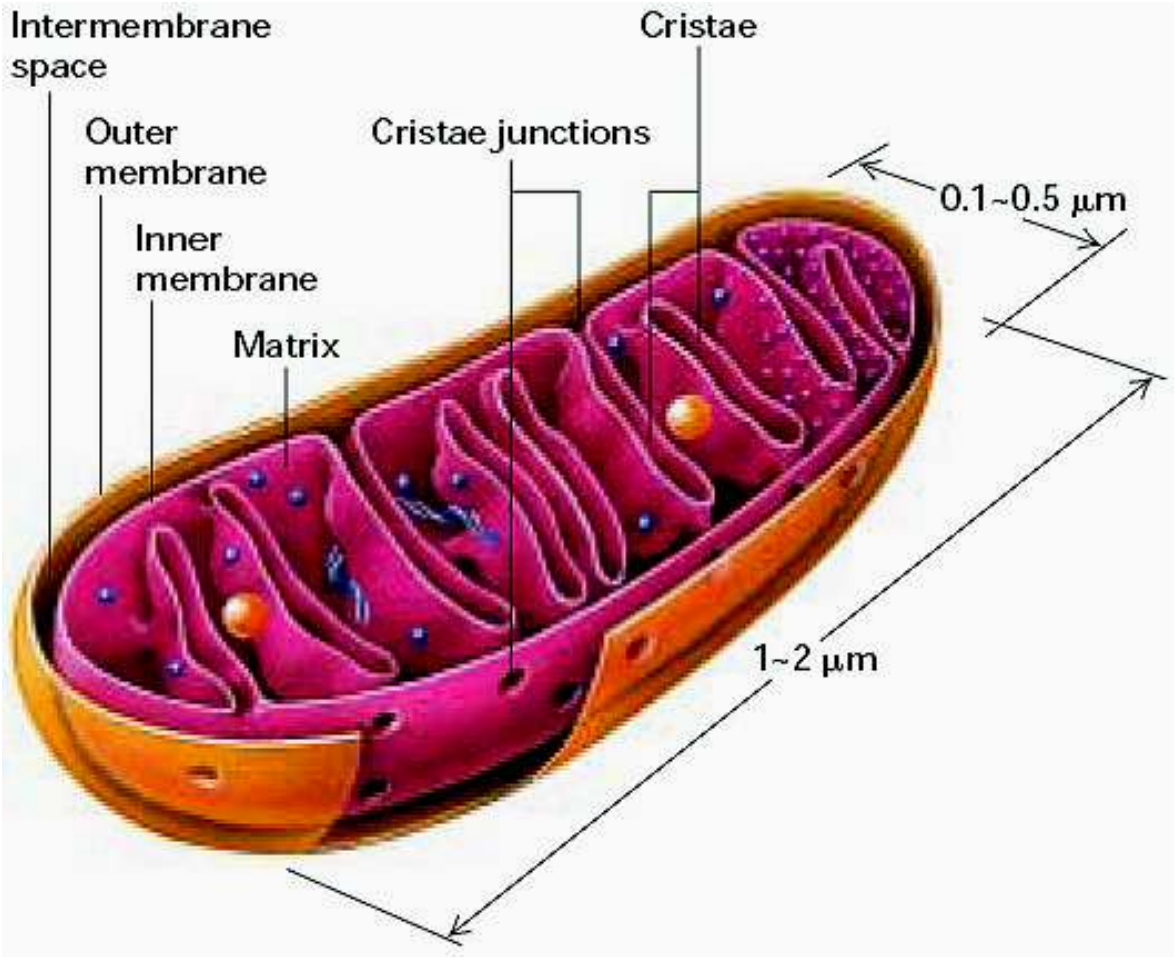
- Occupy a substantial portion of the cytoplasmic volume of eucaryotic cells;
- Essential for the evolution of complex animals.
  - Without mitochondria - anaerobic glycolysis for all ATP.
- In mitochondria, the metabolism of sugars is completed:
  - the pyruvate is oxidized by O_2 to CO_2 and H_2O.
- 15 times more ATP than by glycolysis alone.

The general organization of a mitochondrion



- In the liver, an estimated 67% of the total mitochondrial protein is located in the matrix, 21% is located in the inner membrane, 6% in the outer membrane, and 6% in the intermembrane space. As indicated below, each of these four regions contains a special set of proteins that mediate distinct functions.
- (Courtesy of Daniel S. Friend.)

Internal Structure of a Mitochondrion



The principal membranes and compartments.

The **cristae** form sheets and tubes.

The **intermembrane space** appears continuous with the lumen of each crista.

The **F0F1 complexes** (small red spheres), which synthesize ATP, are intramembrane particles.

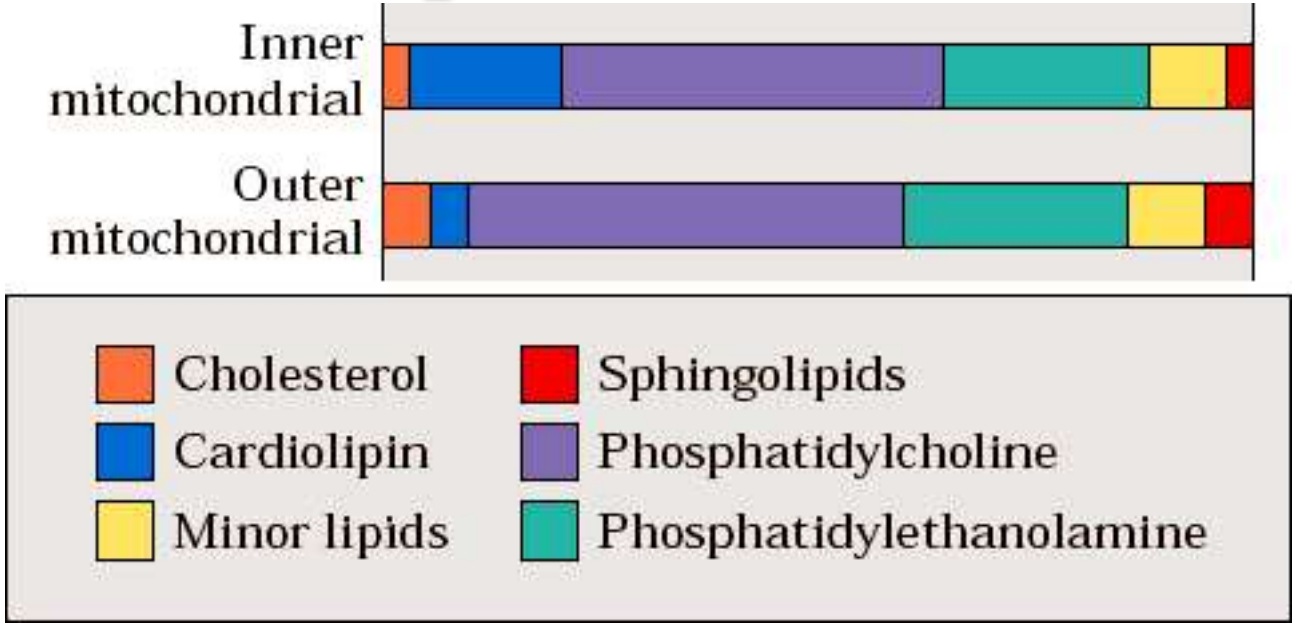
The **matrix** contains the mitochondrial DNA (blue strand), ribosomes (small blue spheres), and granules (large yellow spheres).

The Enzymes of Mitochondrial Membranes

- Outer membrane
  - Monoaminoxidase
  - Fatty acid elongase
  - Choline phosphotransferase
  - Phospholipase A
- Matrix
  - TCA enzymes
  - Fatty acid β-oxidation enzymes
  - Pyruvate carboxylase
  - Gltamate dehydrogenase

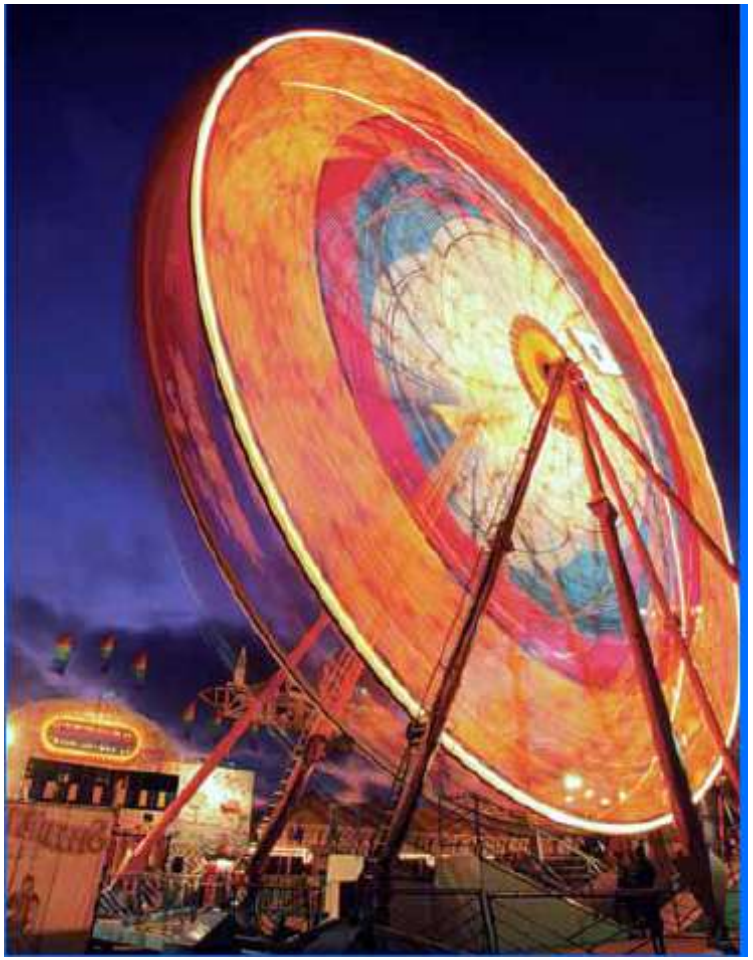
- Inner membrane
  - NADH dehydrogenase
  - Succinate dehydrogenase
  - Cytochromes *b*, c_1, *c*, *a*, a_3
  - Carnitine acyl transferase
  - ADP-ATP translocase
  - Phosphate translocase
  - Glutamate-aspartate translocase
  - Glutamate-OH^--translocase
  - Pyruvate translocase
  - Malate-citrate translocase
  - Malate-α-ketoglutarate translocase

Membrane Composition: Lipid fractions



- Inner membrane contains proteins 70 % and lipids 30 %.
- Specific phospholipid is **cardiolipin**.
- Low cholesterol and sphingolipids content.

Tricarboxylic Acid Cycle



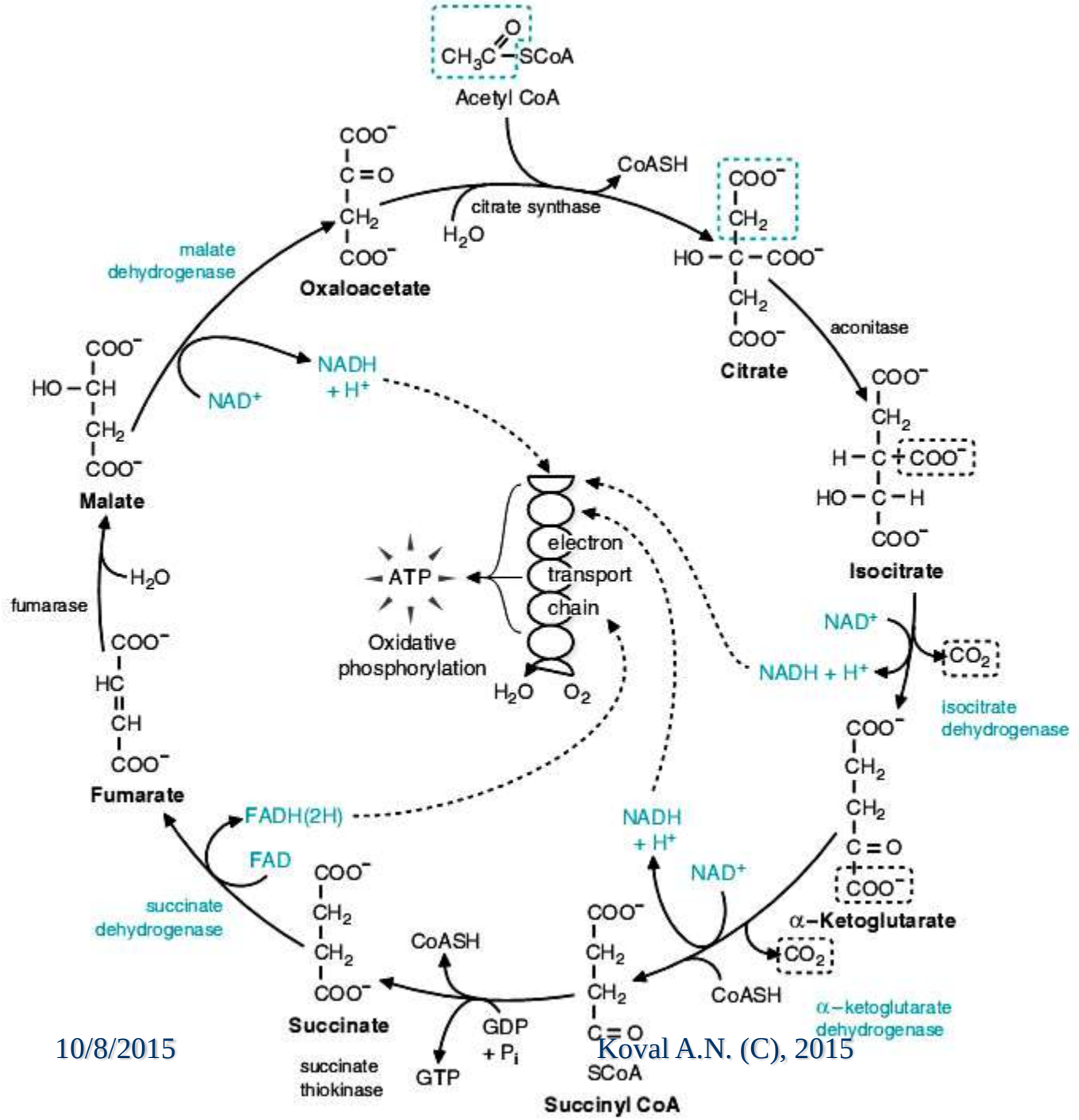
- A time-lapse photograph of a ferris wheel at night. Aerobic cells use a metabolic wheel—the tricarboxylic acid cycle—to generate energy by acetyl-CoA oxidation.
- (Ferns Wheel, DelMar Fair © Corbis/Richard Cummins)

Krebs' Cycle

Hans Adolf Krebs, 1937.

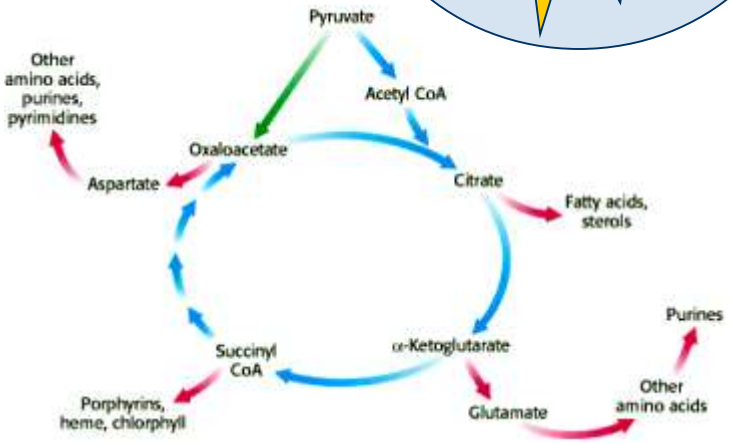
TCA is the common pathway for the final oxidation on all metabolic fuels.

The TCA reactions occurs in mitochondrial matrix.



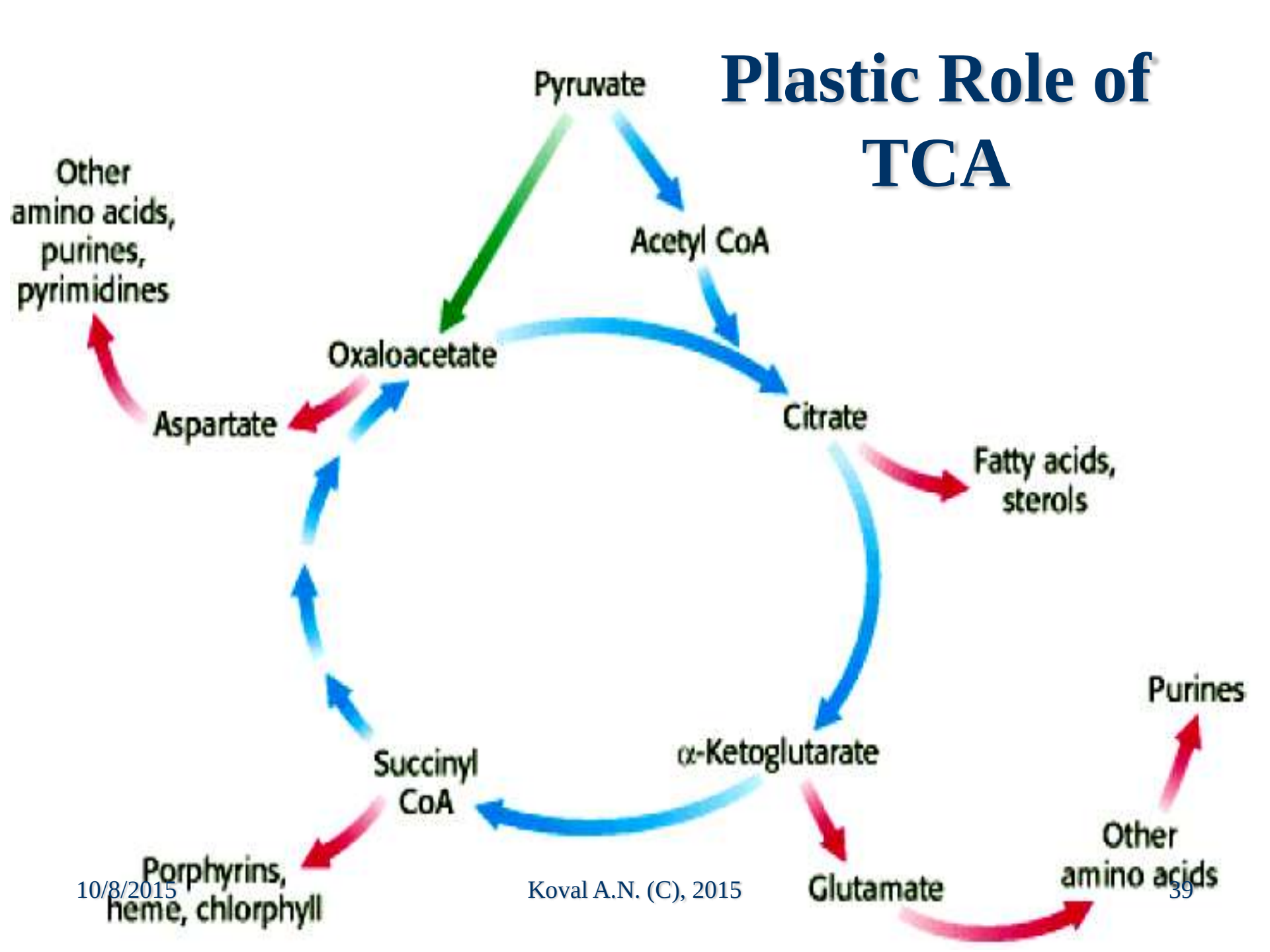
Role of TCA

- Energetic.
  - 1 TCA turn = 12 ATP.
- Plastic.
  - α-KG → glu.
  - OA → asp.
  - Succinyl-CoA → heme.

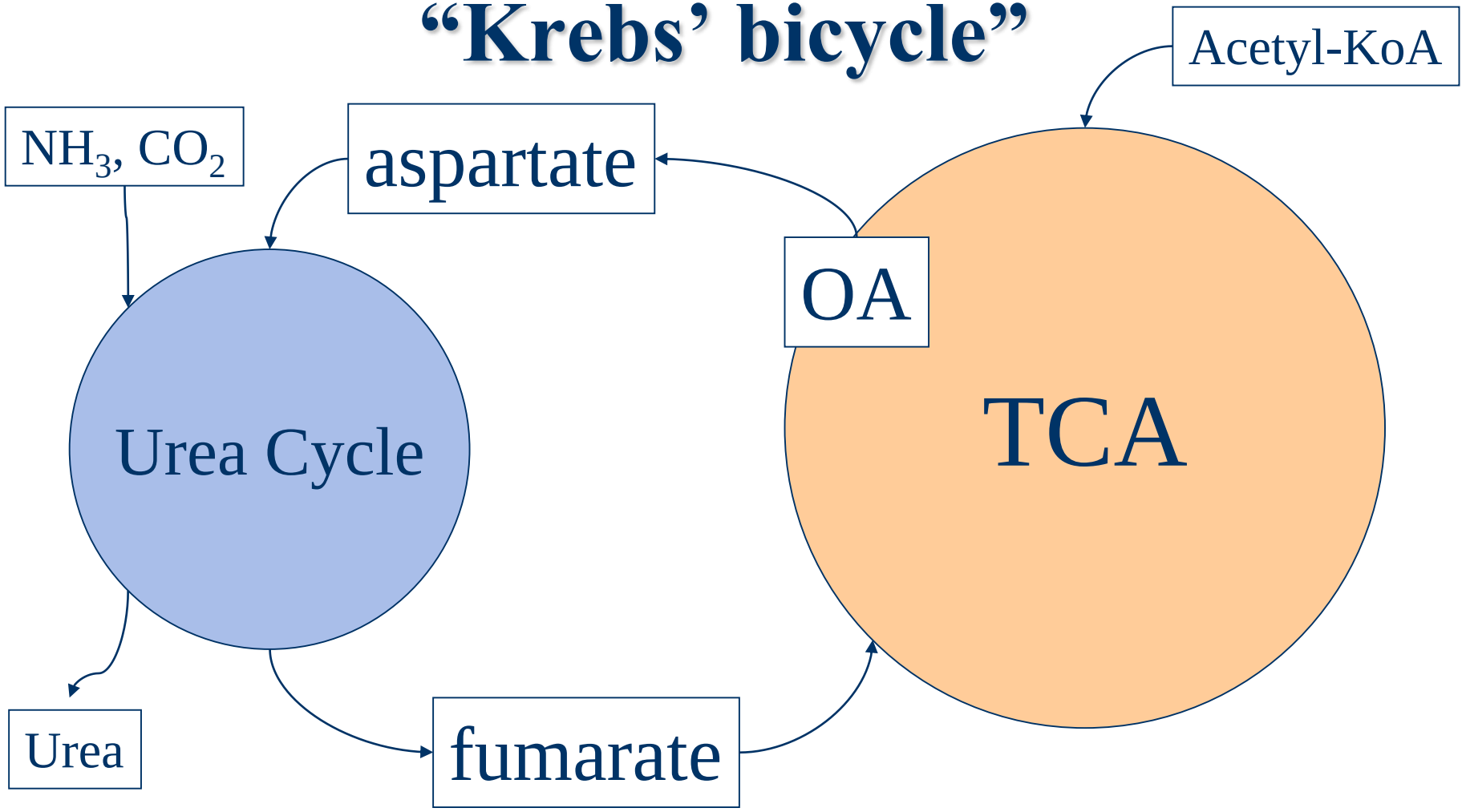


- Regulatory.
  - Urea cycle (formation of urea in liver) depends on TCA.

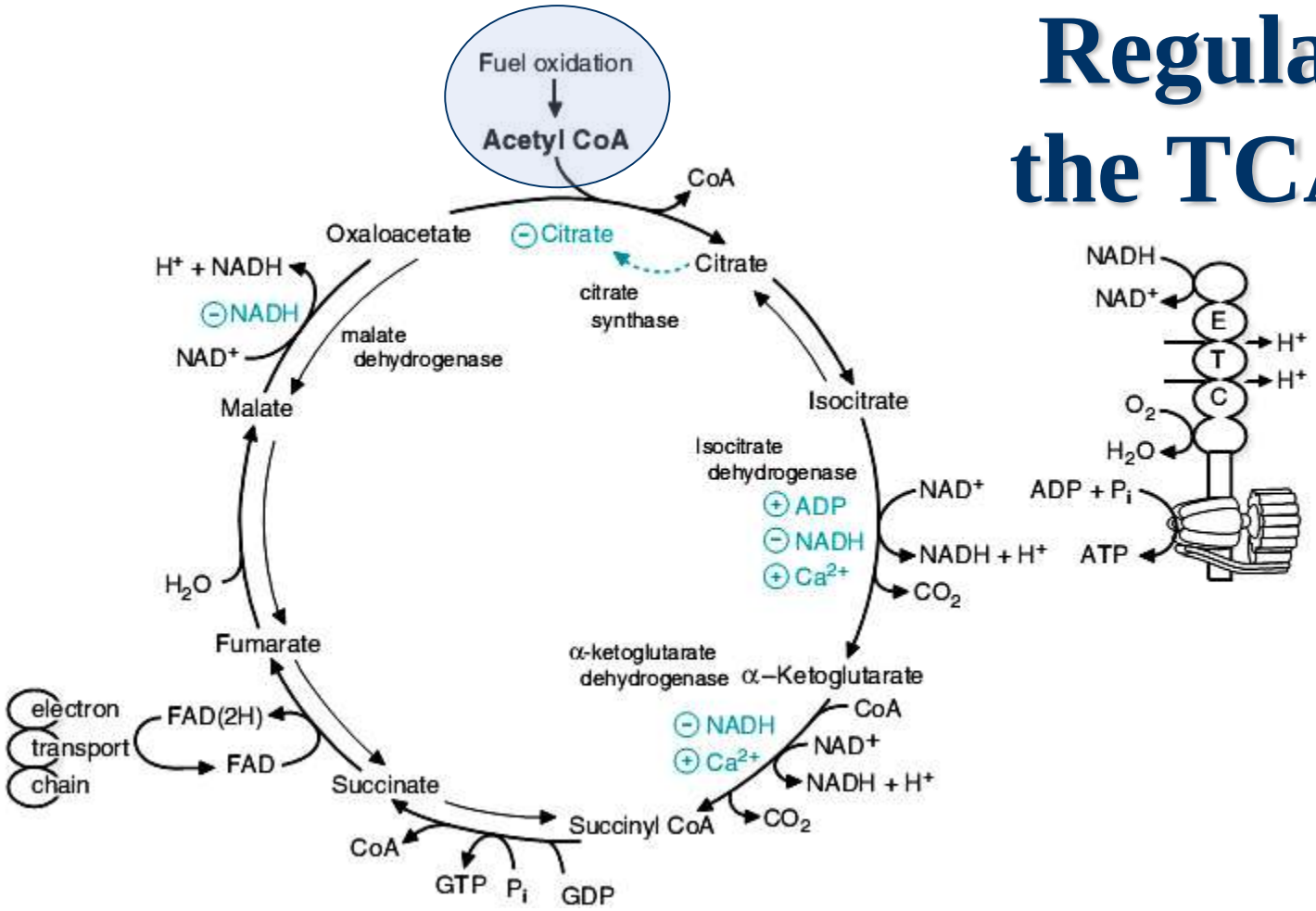
Plastic Role of TCA



Regulatory Role of TCA: "Krebs' bicycle"



Regulation of the TCA Cycle



- At the level of entry of substrates into the cycle,
  - Fuel enters the TCA cycle primarily as acetyl-CoA. The generation of acetyl-CoA from carbohydrates is, a major control point of the cycle.

Regulation of the TCA Cycle (cont'd)

- At the key reactions of the cycle.
  - 3 reactions of the TCA cycle utilize NAD^+ as cofactor ➔ the cellular ratio of NAD^+/NADH has a major impact on the flux of carbon through the TCA cycle.
  - Substrate availability. **Citrate synthase** reaction depends on availability of oxaloacetate.
  - Product inhibition also controls the TCA flux, e.g. citrate inhibits **citrate synthase**, **α-KGDH** is inhibited by NADH and succinyl-CoA.
  - The **key enzymes** of the TCA cycle are also regulated allosterically by Ca^{2+}, ATP and ADP.

Inhibitors of Krebs Cycle

Enzyme	Inhibitor
Aconitase	Fluoracetate (non-competitive)
α-Ketoglutarate dehydrogenase complex	Arsenite (non-competitive)
Succinate dehydrogenase	Malonate (competitive)

Conclusion

- Biologic systems use chemical energy to power living processes.
- ATP acts as the "energy currency" of the cell.
- Nutrients (carbohydrates, fats and proteins) should be “processed” before catabolizm in Krebs cycle.
- **Krebs cycle** (tricarboxylic acid cycle, TCA) is the important aerobic metabolism in our cells.
- … but ATP is not produced in TCA! Why?!

Biologic Oxidation

2

Tissue respiration.
Oxidative phosphorylation.
Microsomal oxidation and peroxidation

Alexander KOVAL
PhD, senior lecturer

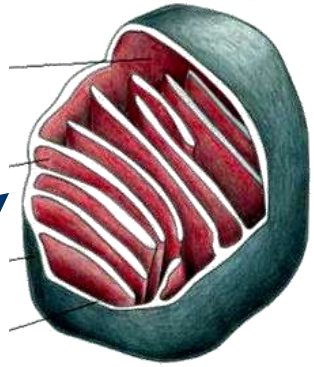
Introduction

- *… but ATP is not produced in TCA! Why?!*
- The energy of TCA is the **reducing energy** of electron carriers – coenzymes **$NADH+H^+$** and **$FADH_2$**.
- By the way! Oxygen is not necessary for TCA! How do you like this?
- Guess what? We are a bit **ROBOTS**! **Electric currents are inside us!**
- And this current is in Electron-transporting chains in mitochondria and microsomes.

Content

- The Ways of Oxygen Consumption in the Organism
- Structure & Functions of Respiratory Chain
  - Oxidative Phosphorylation
- Microsomal Oxidation. Peroxydase Pathway. Monooxygenase Systems. Dioxygenase System
- Free Radicals, Peroxidation and Antioxidants

The Ways of Oxygen Consumption in the Organism



Mitochondrial respiration **90-95%**

O_2

Microsomal oxidation **5-10%**

up to 40% in liver

Peroxidation **2-5%**

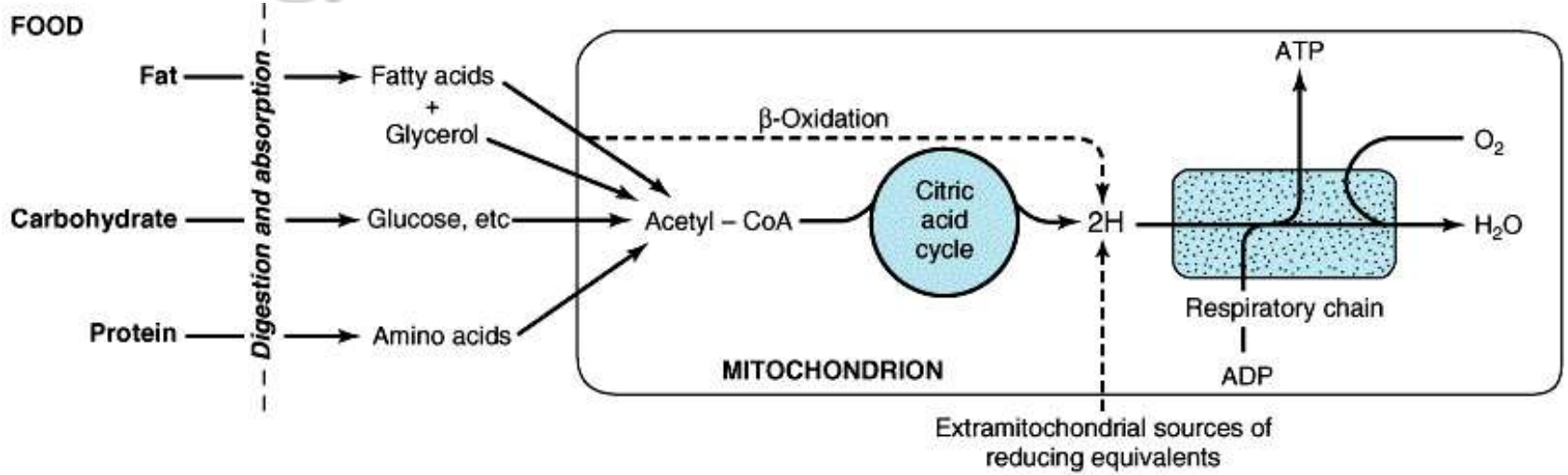
Biologic Oxidation (BO)

- **Oxidation** is the removal of electrons and **reduction** – the gain of electrons.
  - Biologic oxidation can take place without molecular oxygen.
- **Respiration** is the process by which cells gain energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water.
  - Tissue respiration.

Biomedical Importance of BO

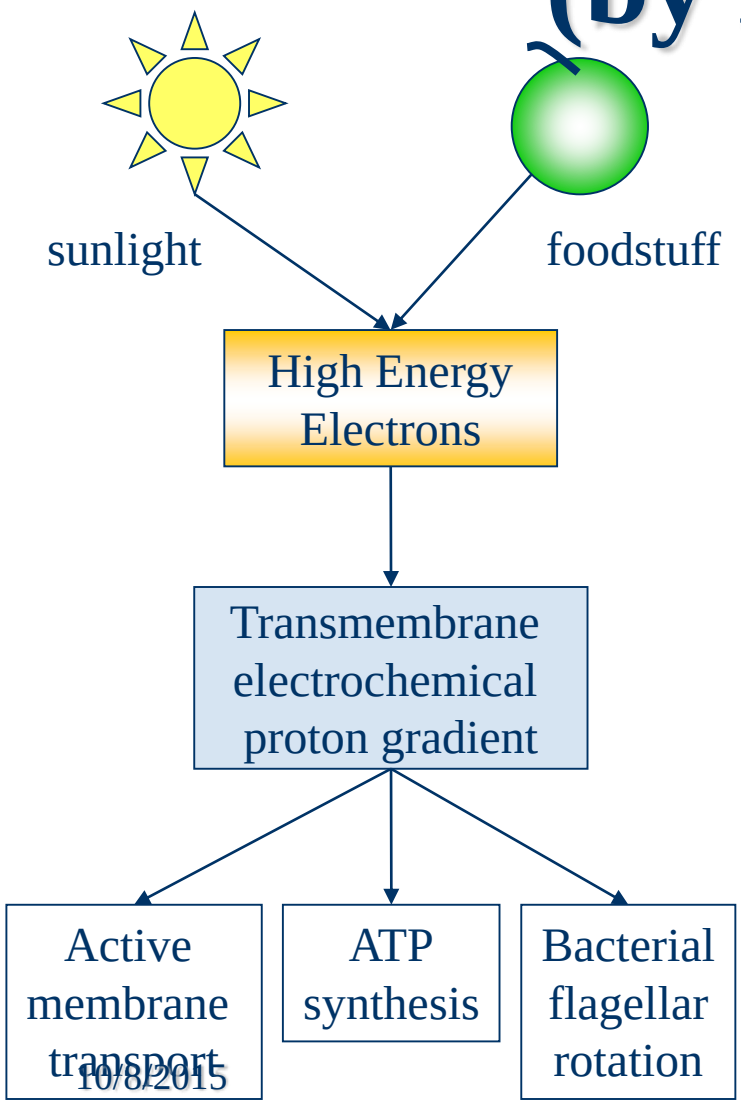
- O_2 is incorporated into a variety of substrates by enzymes designated as **oxygenases;**
  - many drugs, pollutants, and chemical carcinogens (**xenobiotics**) are metabolized by enzymes of this class, known as the **cytochrome P450 system.**
- Administration of oxygen can be lifesaving in the treatment of patients with respiratory or circulatory failure.

Energy Conversion: Mitochondria



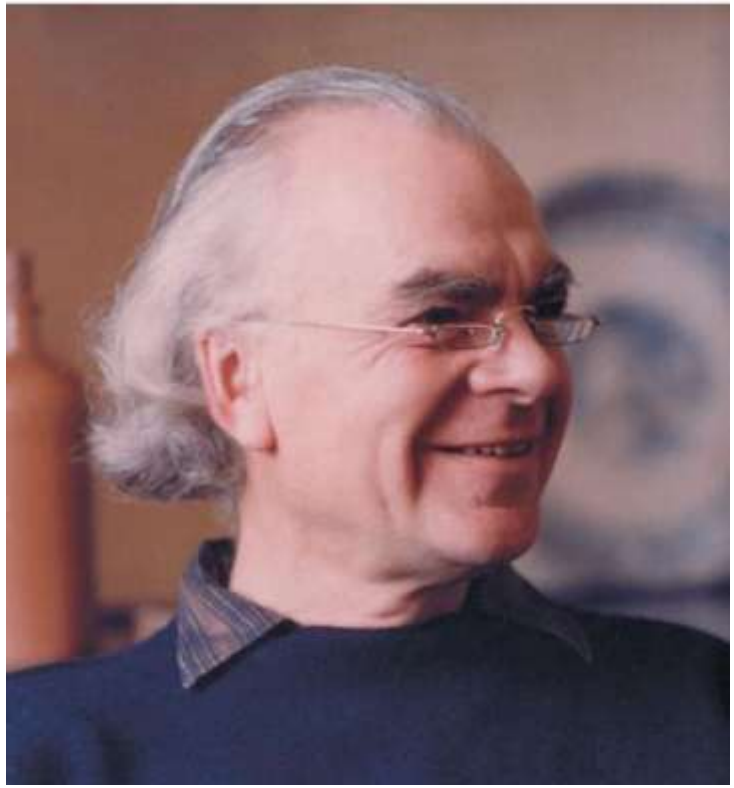
- After the cytosolic stage of biologic oxidation, energy derived from the partial oxidation of energy-rich carbohydrate molecules is used to form ATP.
- Energy generation occurs more efficiently on membranes.
  - In the aerobic respiration that enables us to use oxygen to produce large amounts of ATP from food molecules.

Chemiosmotic Coupling (by P.Mitchell)



- The common pathway used by mitochondria, chloroplasts, and prokaryotes to harness energy for biological purposes operates by a process known as **chemiosmotic coupling** – reflecting a link between the chemical bond-forming reactions that generate ATP ("chemi") and membrane-transport processes ("osmotic").
- The coupling process occurs in two linked stages, both of which are performed by protein complexes embedded in a membrane.

Peter Mitchell (1920–1992)



- Mitchell was awarded the Nobel Prize in Chemistry in 1978 “for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory.”
  - In 1963 Mitchell resigned from his position at Edinburgh University in Scotland and in 1965 he set up a private research institute with his long-time friend and collaborator, Jennifer Moyle.
  - They continued to work on bioenergetics in a laboratory in Mitchell’s home, Glynn House, in Cornwall (UK).

Electron Transporting Chain, ETC

- The mitochondria contain the series of catalysts known as the **respiratory chain (electron transporting chain, ETC)** that **collect** and **transport** reducing equivalents and direct them to their final reaction with oxygen to form **water**.
- ETC components are imbedded to the inner membrane of mitochondria.

Principles of ETC Functioning

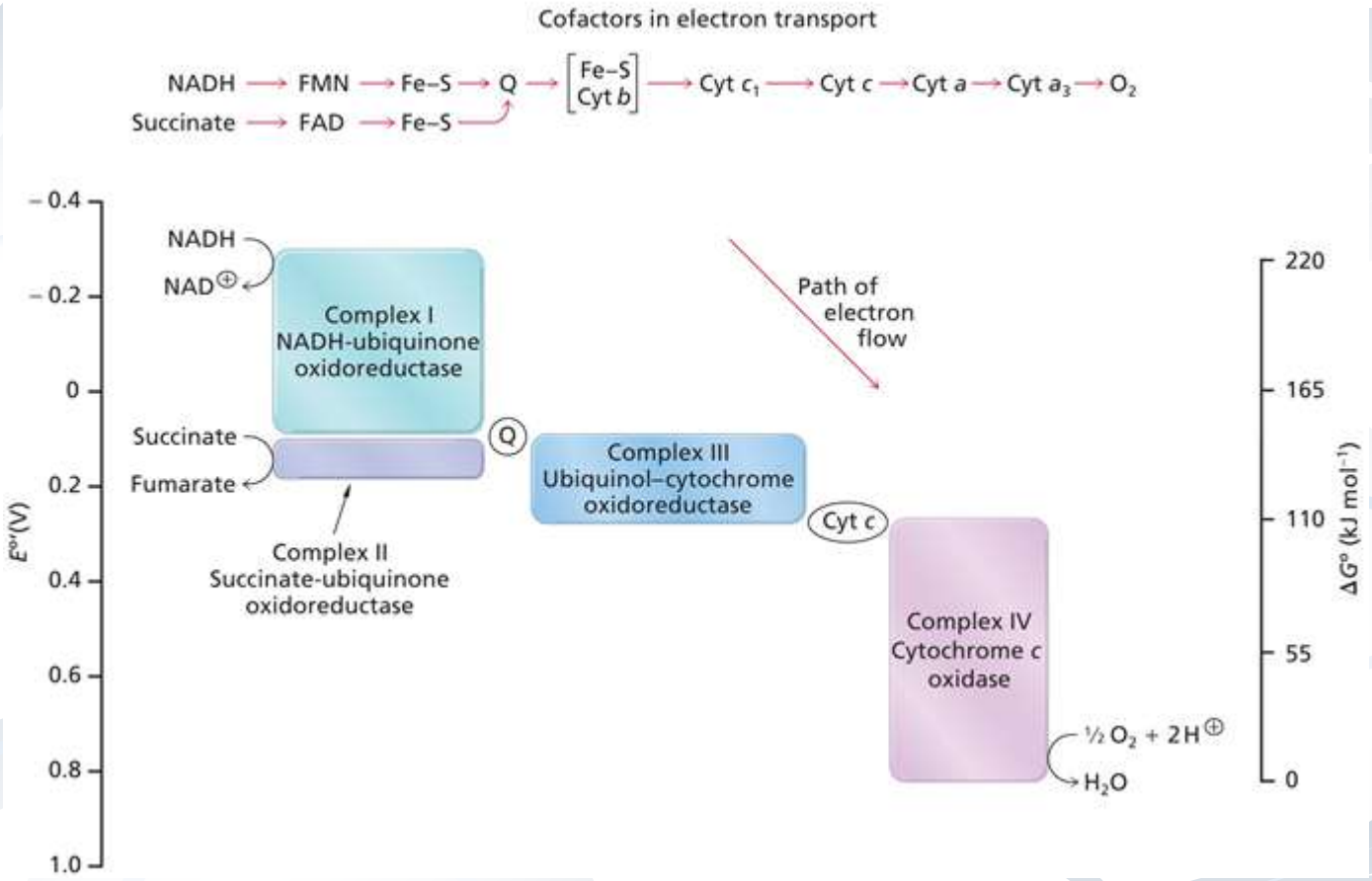
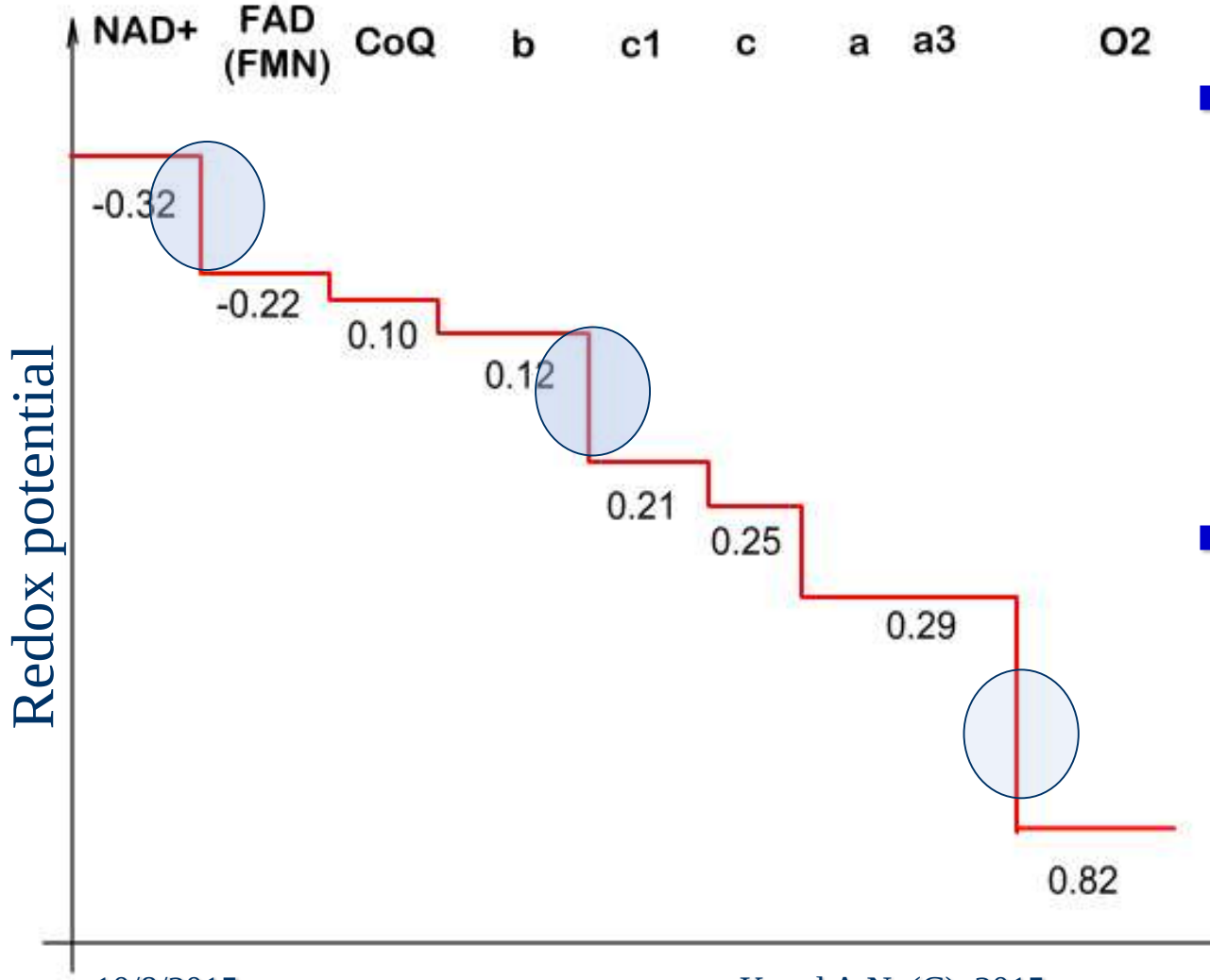


Table 14.1 Standard reduction potentials of mitochondrial oxidation–reduction components

Substrate of Complex	$E^{o\prime}$ (V)
NADH	−0.32
Complex I	
FMN	−0.30
Fe–S clusters	−0.25 to −0.05
Succinate	+0.03
Complex II	
FAD	0.0
Fe–S clusters	−0.26 to 0.00
QH_2/Q	+0.04
$(\cdot Q^{\ominus}/Q$	−0.16)
$(QH_2/\cdot Q^{\ominus}$	+0.28)
Complex III	
Cytochrome b_1.	−0.01
Cytochrome b_H	+0.03
Fe–S cluster	+0.28
Cytochrome c_1	+0.22
Cytochrome c	+0.22
Complex IV	
Cytochrome a	+0.21
Cu_A	+0.24
Cytochrome a_3	+0.39
Cu_B	+0.34
O_2	+0.82

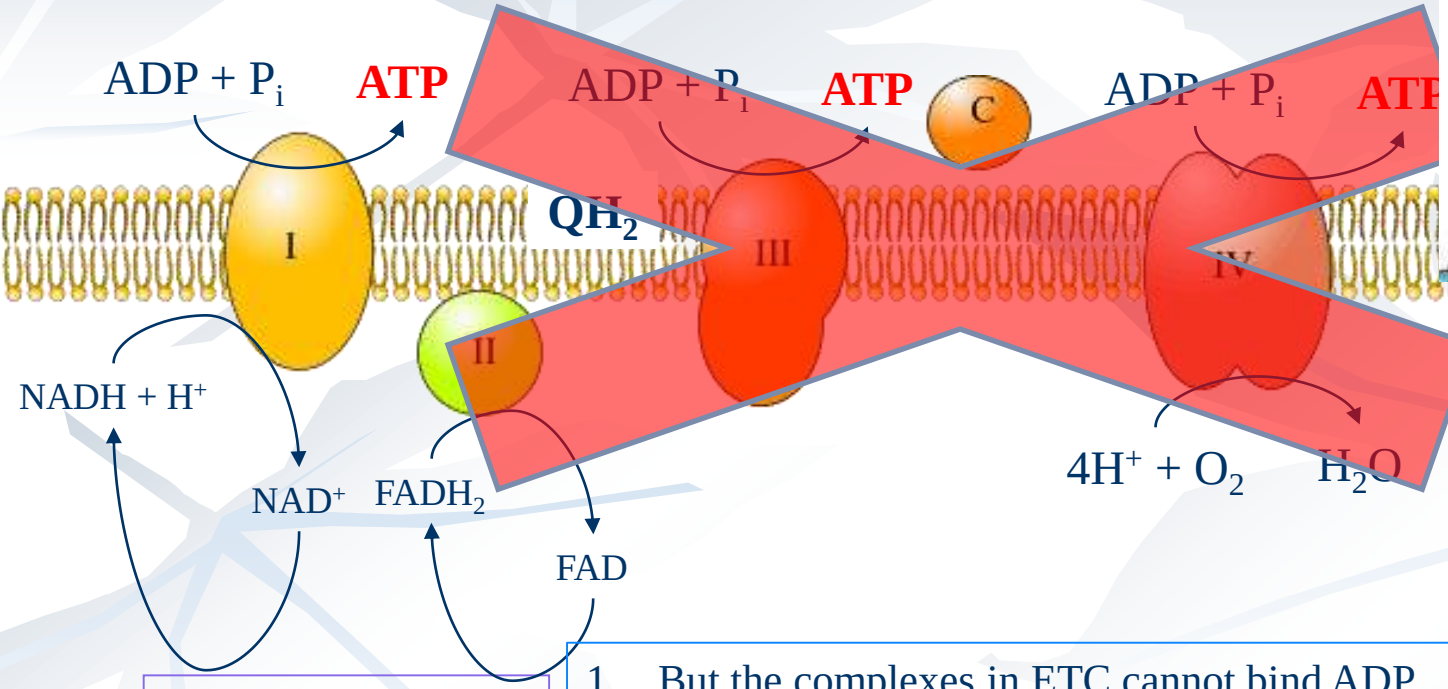
ETC Organization

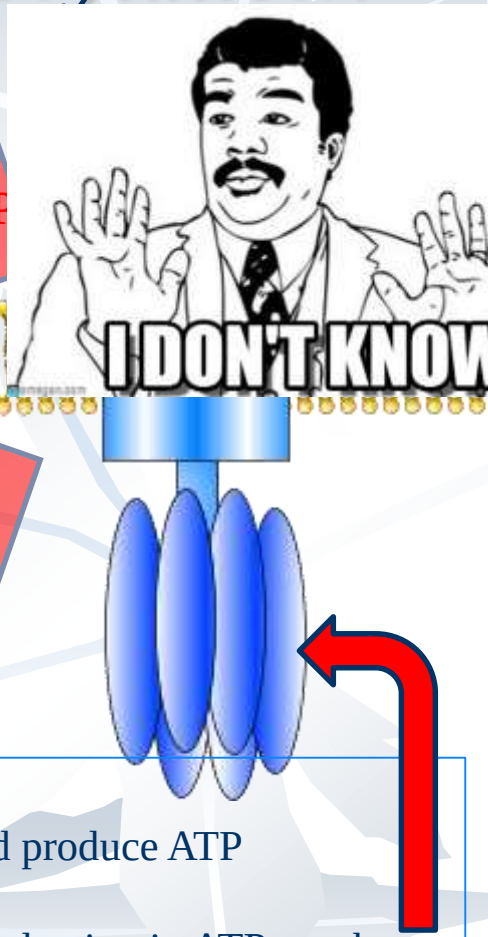


- The components of ETC are arranged according to their redox-potential.
- The “sites of phosphorylation” are circled.

How ATP is produced in mitoETC?

Maybe by substrate-level phosphorylation?

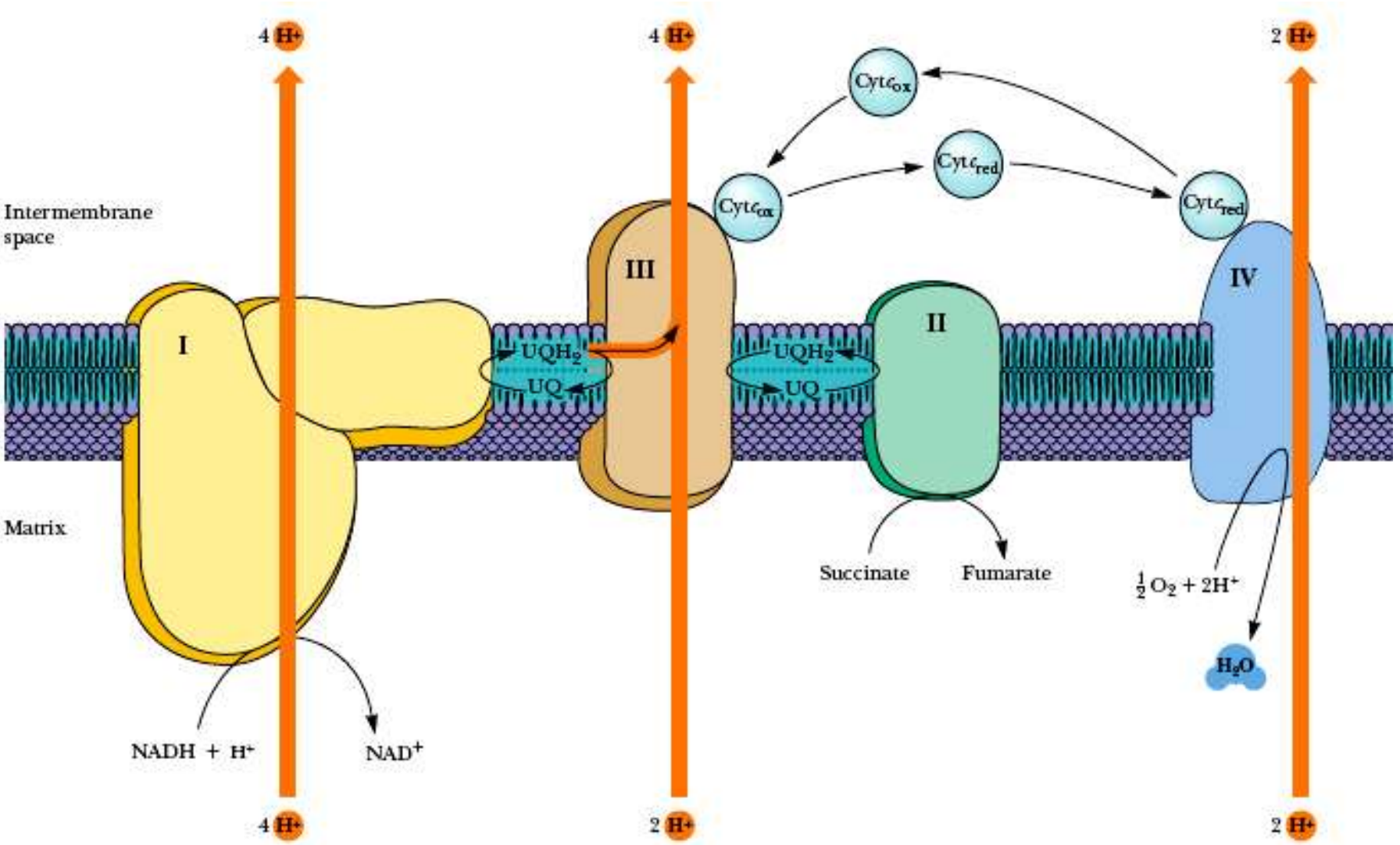




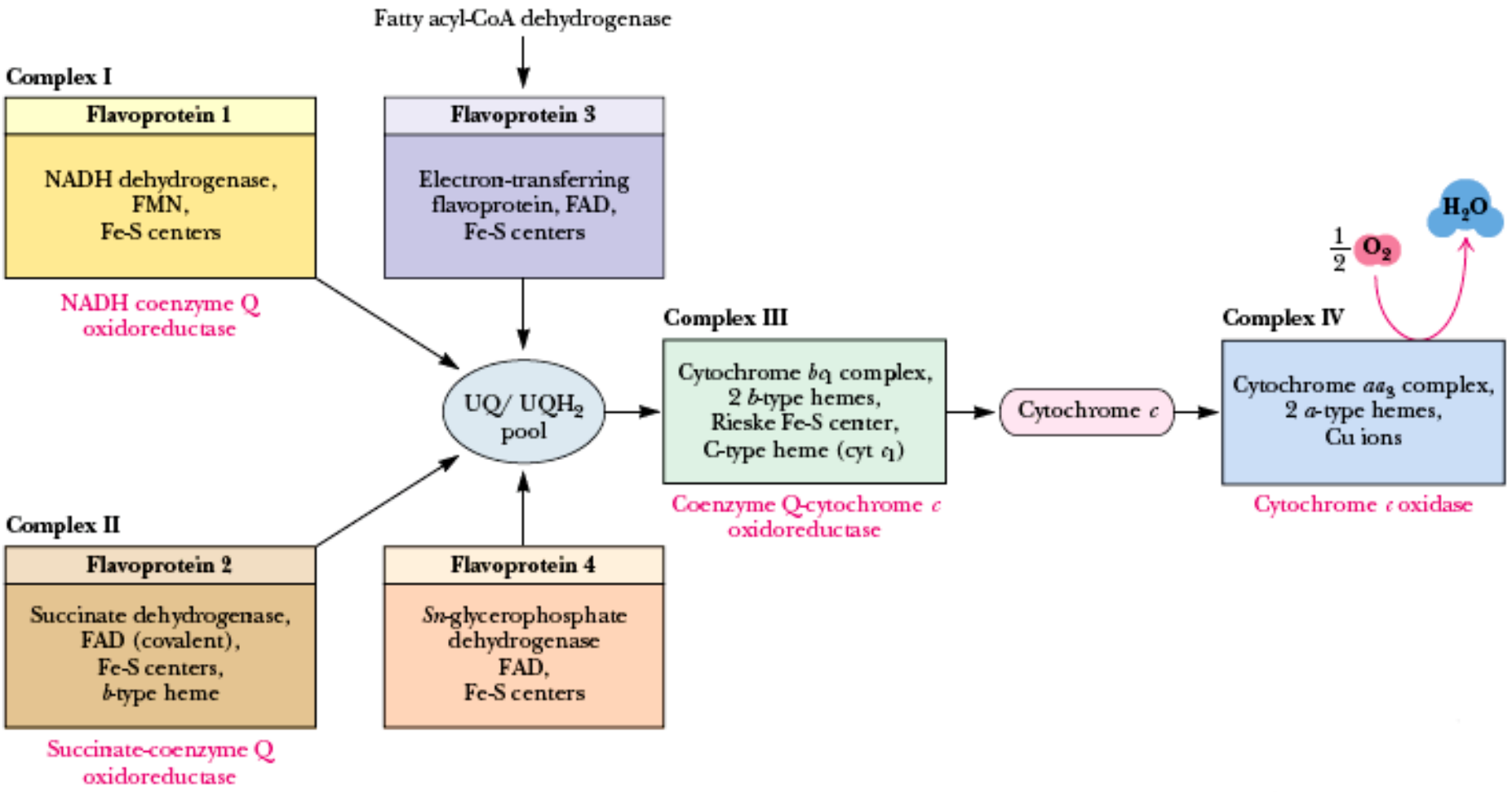
TCA

1. But the complexes in ETC cannot bind ADP.
2. Only ATP-synthase can bind ADP, and P_i, and produce ATP
3. Also ETC can form H^+-gradient.
4. And this H^+-gradient is necessary for ATP production in ATP-synthase.

ETC Functioning (by Mitchell hypothesis)



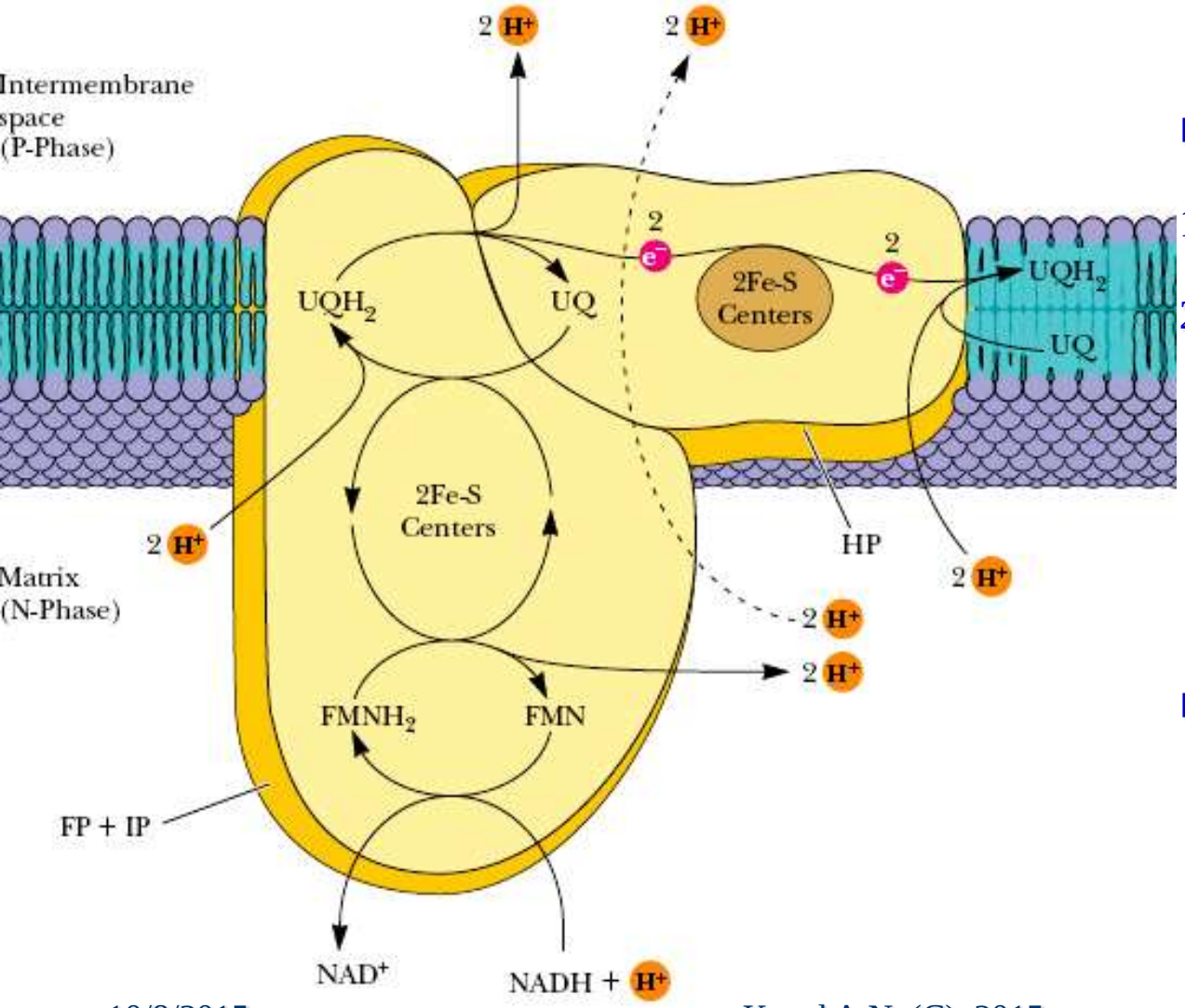
ETC Complexes: Overview



ETC functions

1. It is the final common pathway in aerobic cells.
2. NAD^+ and FAD are reduced to NADH + H^+ and $FADH_2$ respectively in most oxidation reactions. By ETC these coenzymes are reoxidized to NAD^+ and FAD.

Complex I (NADH-CoQ reductase)

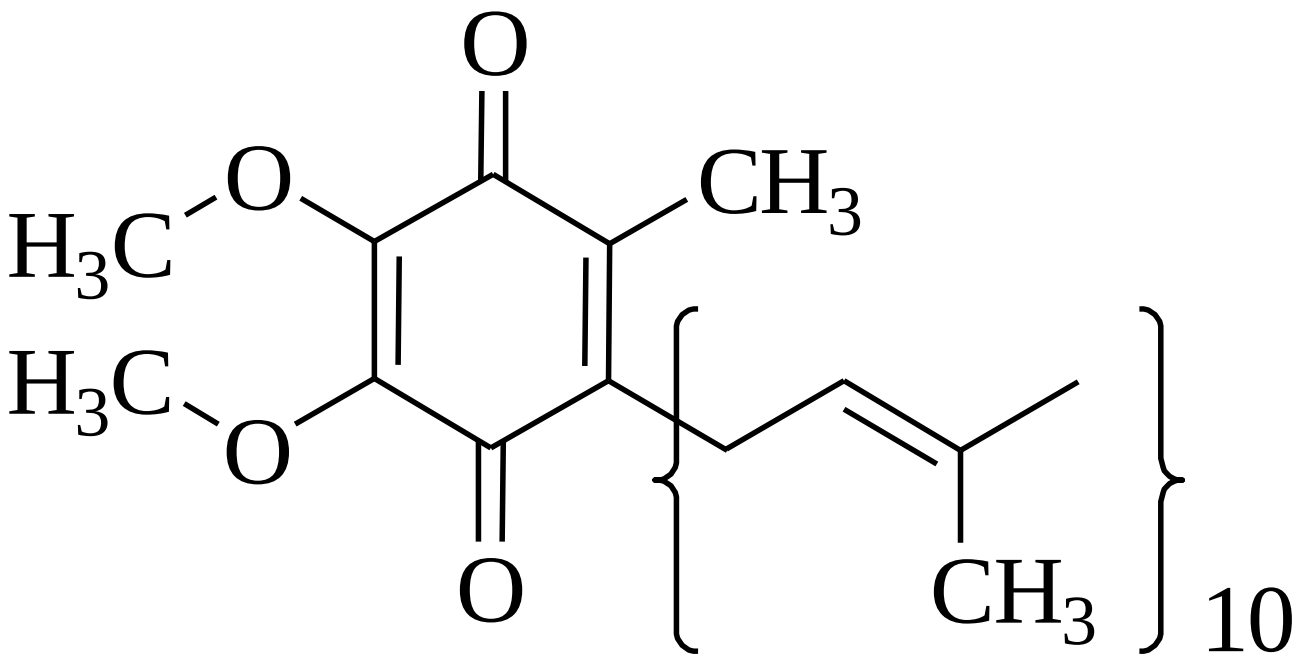


- Contains:

1. FMN
2. FeS centres (22-24 iron-sulfur (Fe-S) proteins in 5-7 clusters).

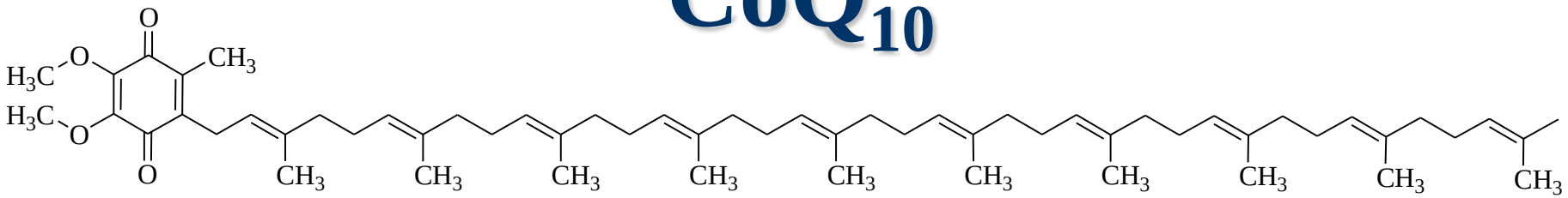
- Electron acceptor is CoQ

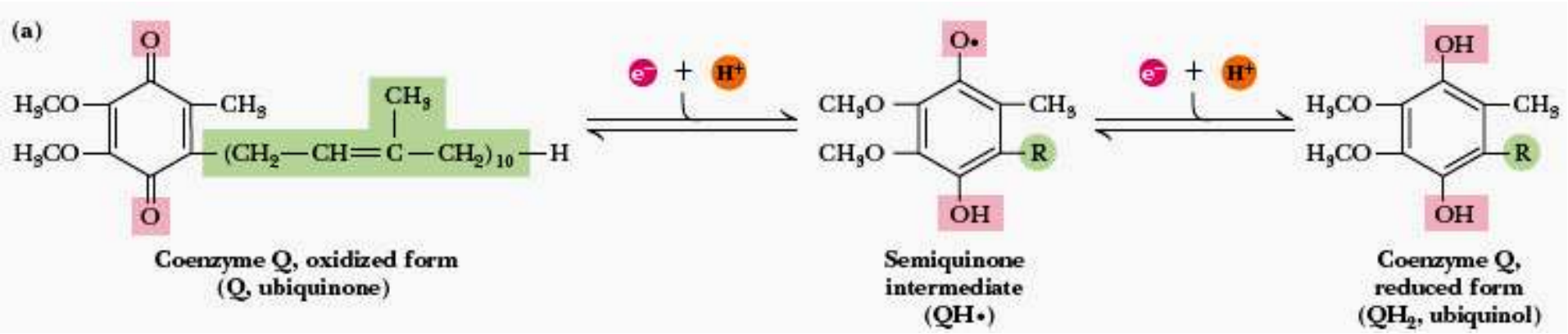
Coenzyme Q (CoQ) or Ubiquinone



- **CoQ** is a component of the inner mitochondrial membrane involved in the process of **electron transport**.
- It draws electrons into the respiratory chain, not only from **NADH** but also from **succinate.**

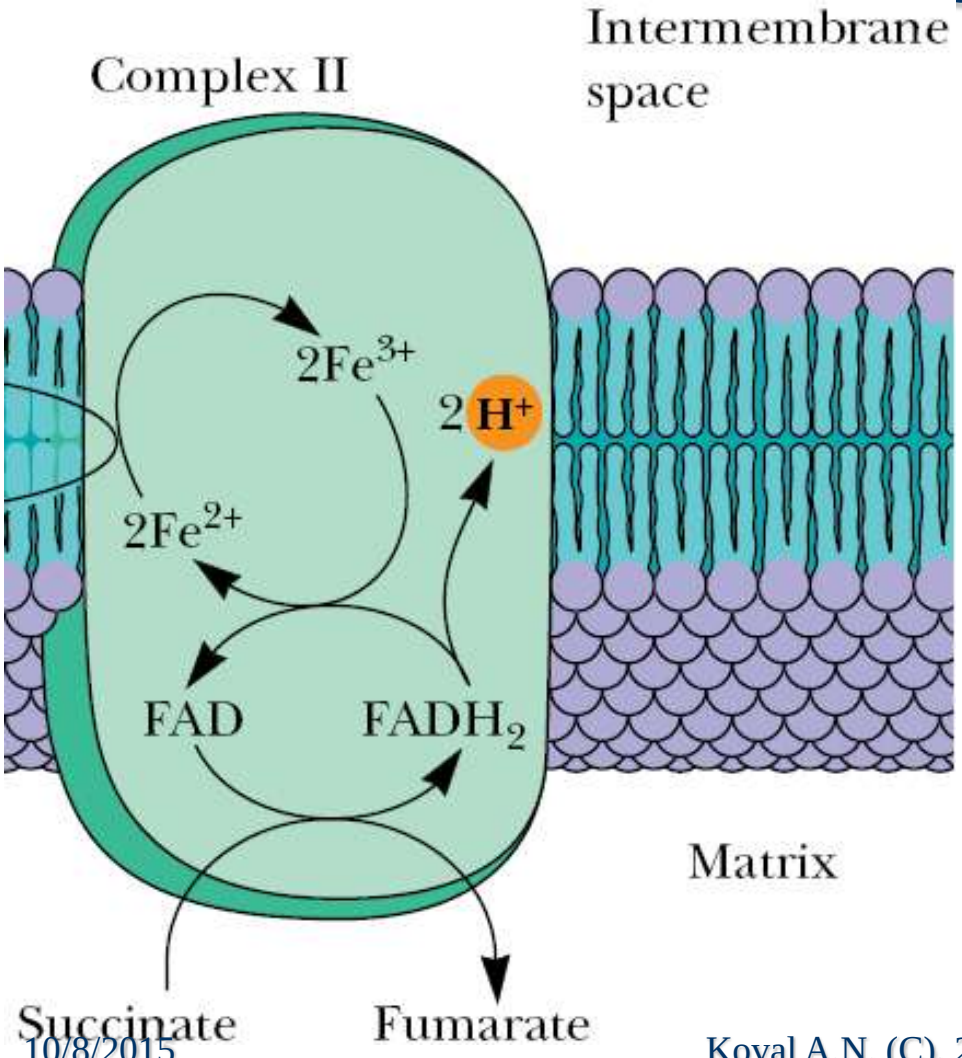
CoQ_{10}





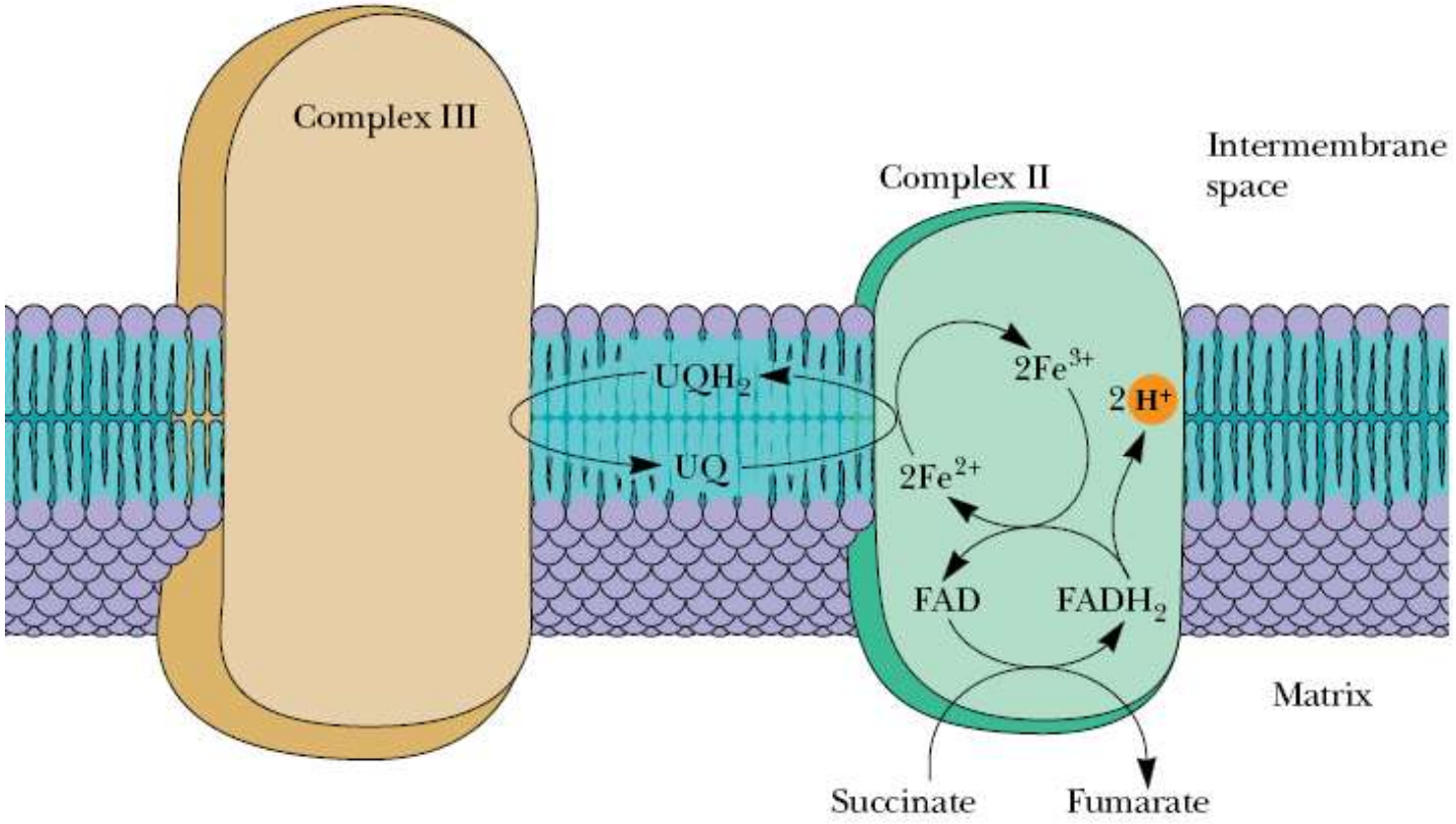
- **CoQ** is oxidized by **cytochromes**, it is a collection point of electrons from several flavoprotein dehydrogenases.

Complex II (Succinate-CoQ reductase)



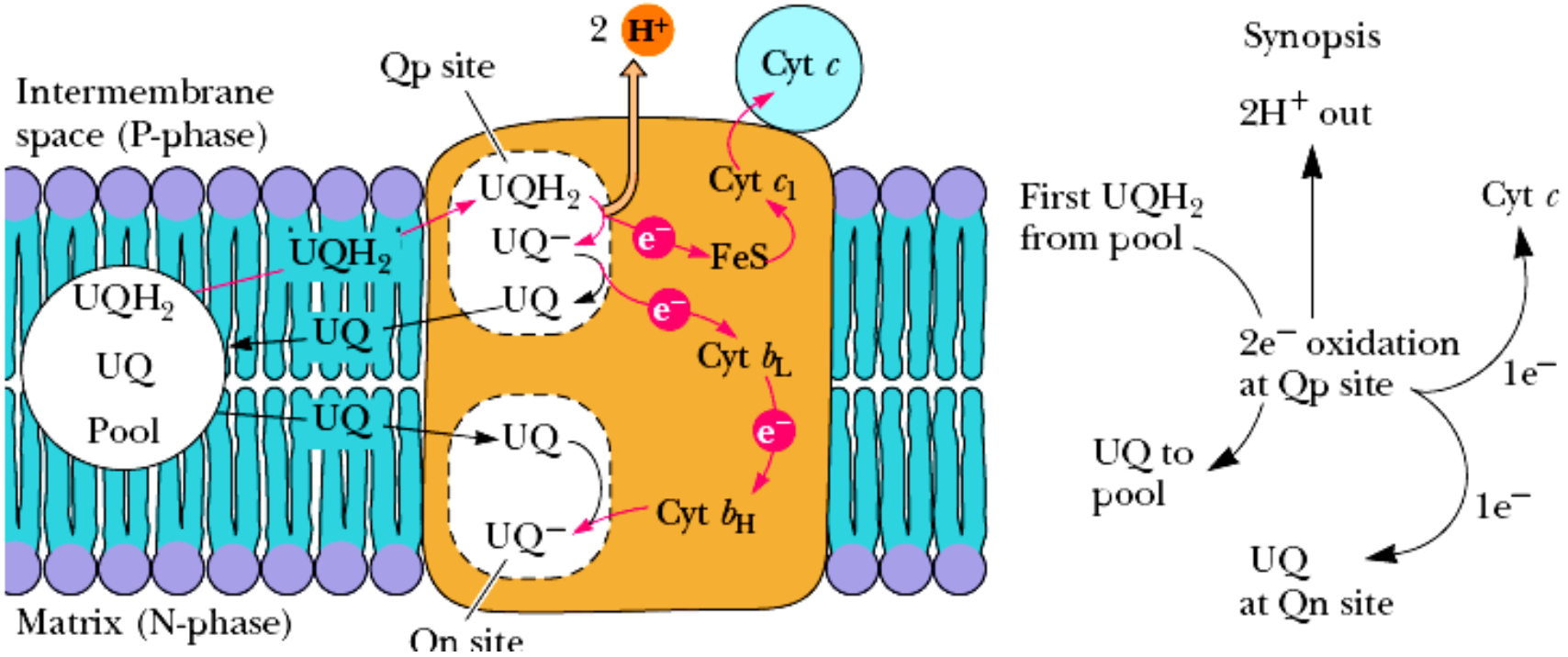
- Complex II contains FAD and 7-8 Fe-S proteins in 3 clusters and cytochrome b_{560}.
- Complex II is perhaps better known by its other name—**succinate dehydrogenase,** the only TCA cycle enzyme that is an integral membrane protein in the inner mitochondrial membrane. This enzyme has a mass of approximately 100 to 140 kD

Complex II and III



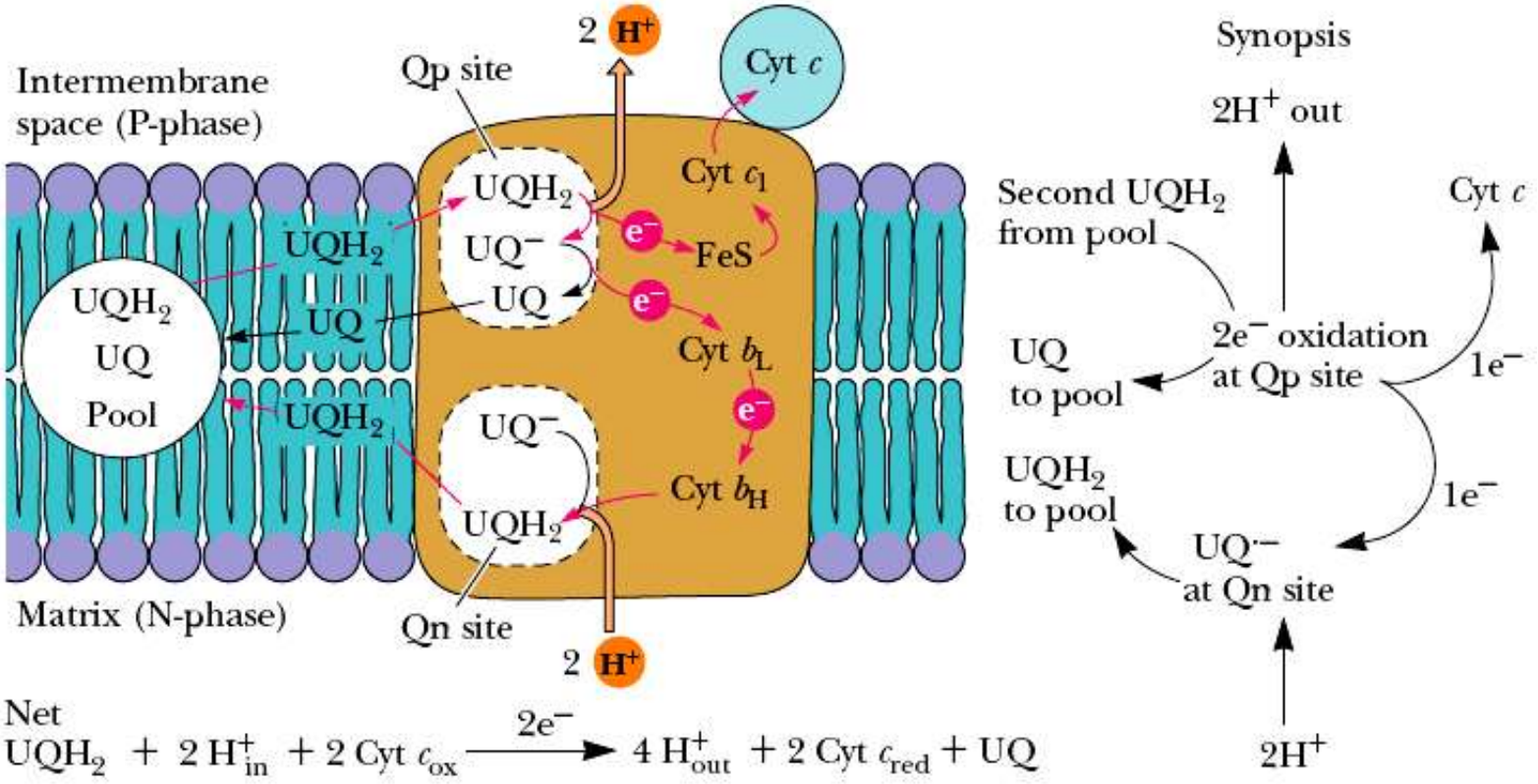
- CoQ accepts electrons from both complex I And complex II and donates electrons to complex III.

Q-cycle (1/2)



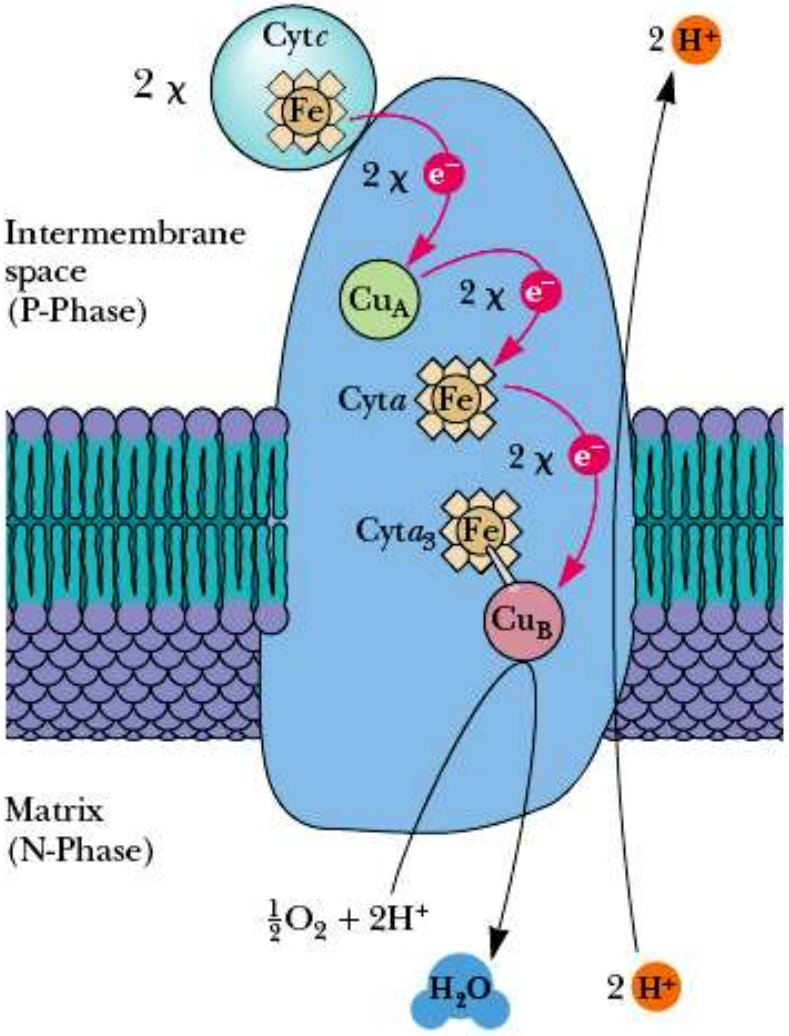
- The electron transfer pathway following oxidation of the first UQH_2 at the Q_p site near the cytosolic face of the membrane.

Q-cycle (2/2)



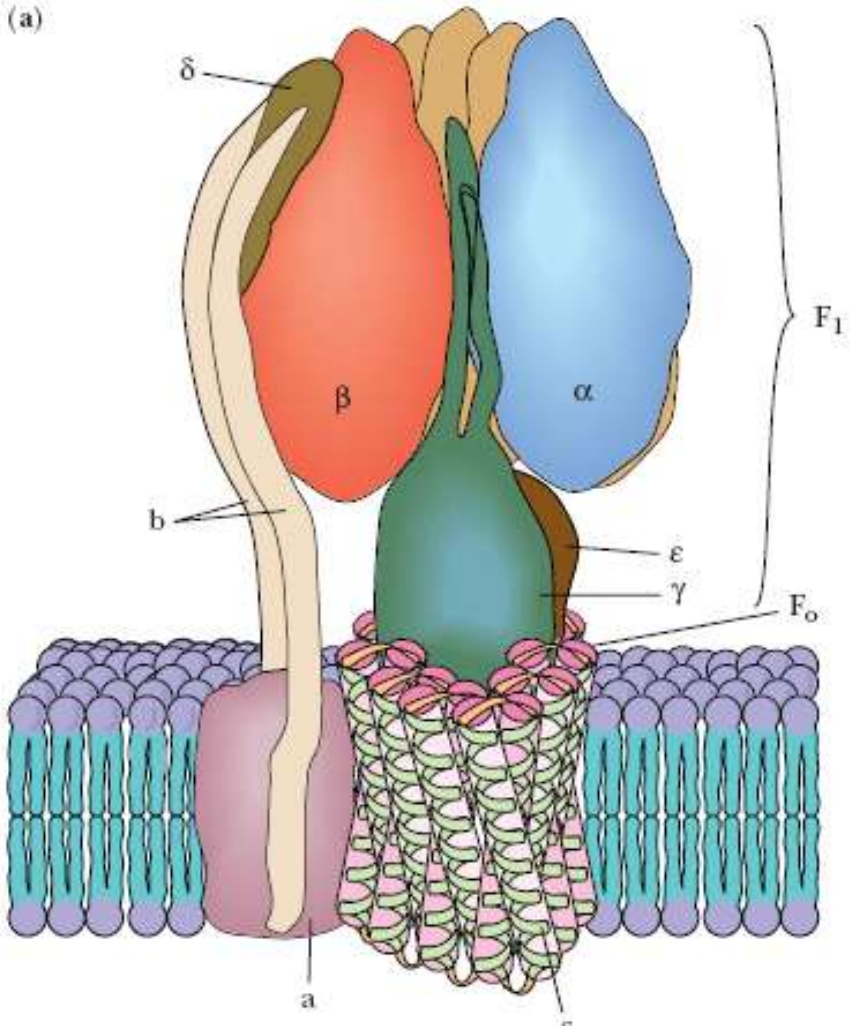
- The pathway following oxidation of a second UQH_2.

Complex IV: Cytochrome *c* Oxidase



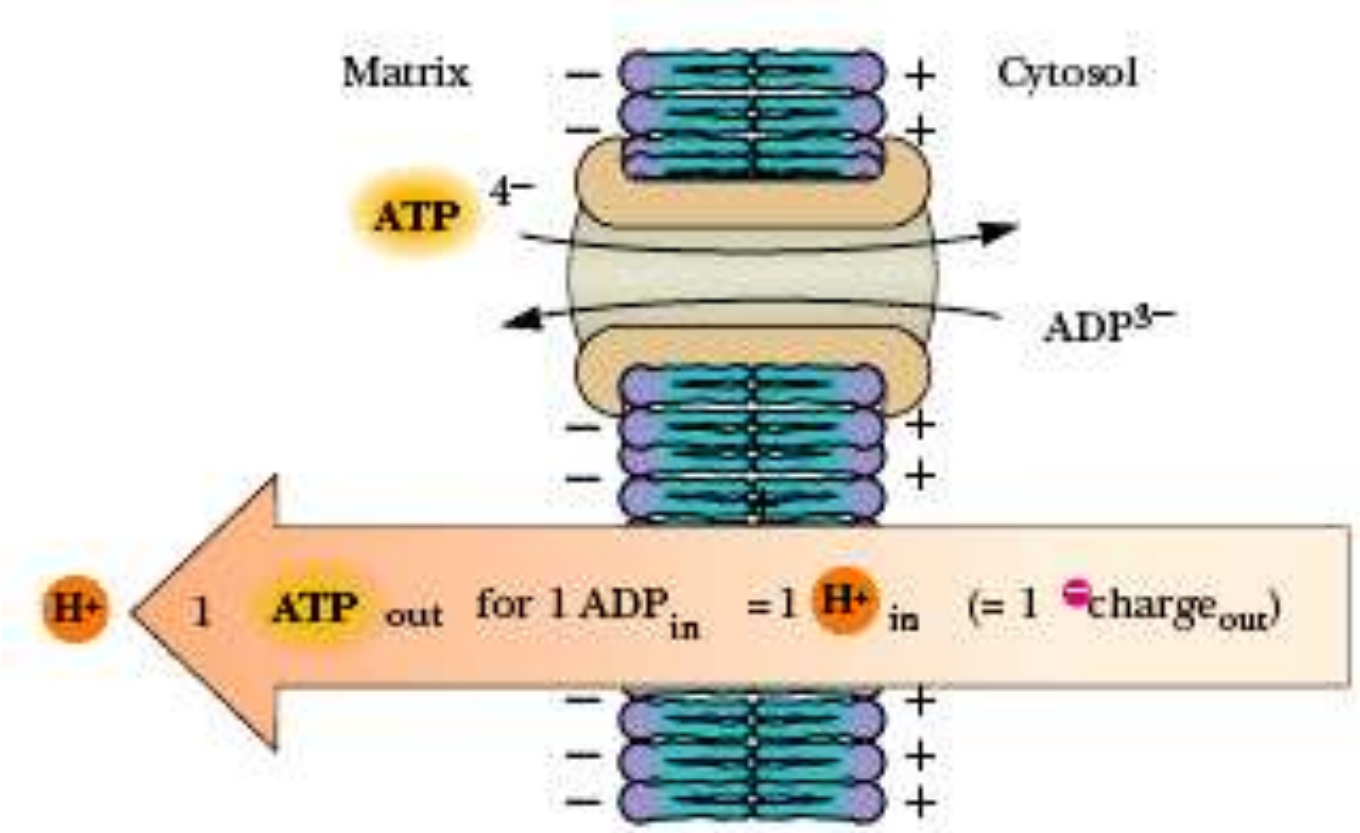
- Complex IV is called cytochrome *c* oxidase because it accepts electrons from cytochrome *c* and directs them to the four-electron reduction of O_2 to form H_2O.

H^+-ATPase



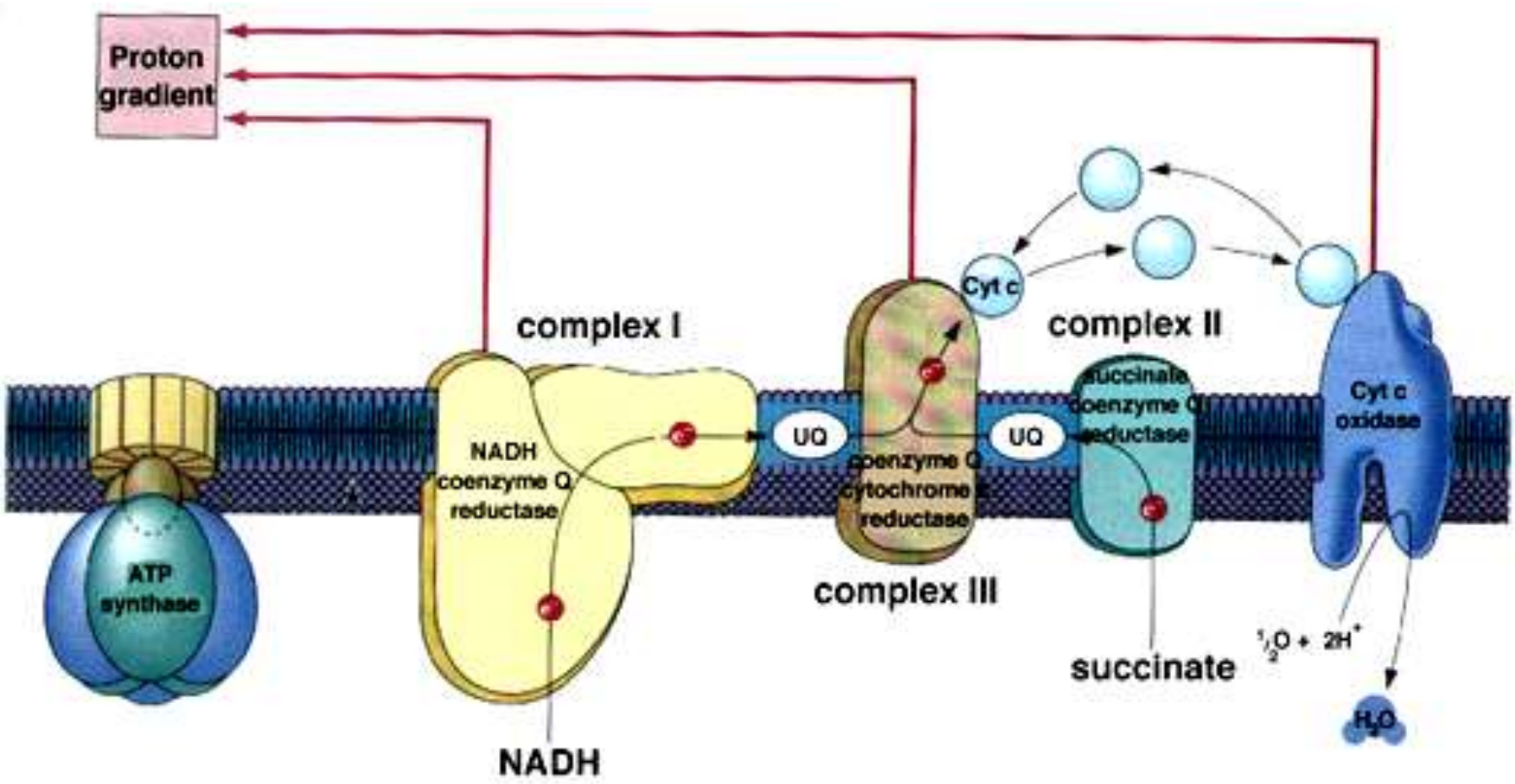
- Ion gradient across a membrane is a form of stored energy, which can do useful work when the ions are flowing back across the membrane.
- H+ flows back down its electrochemical gradient through **ATP synthase**, which catalyzes the synthesis of ATP from ADP and inorganic phosphate (P_i).
  - This ubiquitous enzyme plays the role of a turbine, permitting the proton gradient to drive the production of ATP.

ATP/ADP translocase

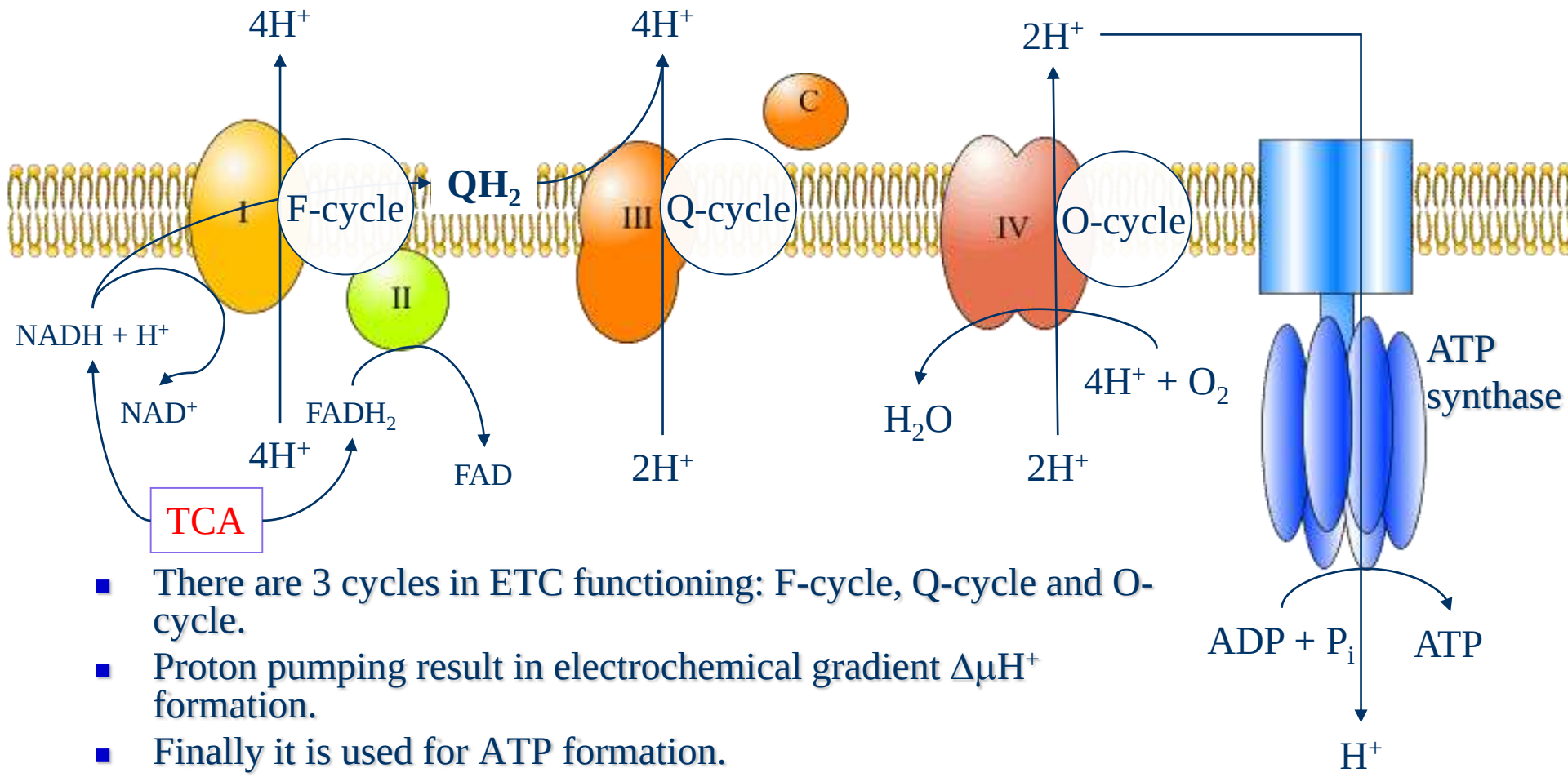


- Outward transport of ATP (via the ATP/ADP translocase) is favored by the membrane electrochemical potential.

Respiratory Chain Functioning



Functional scheme of ETC

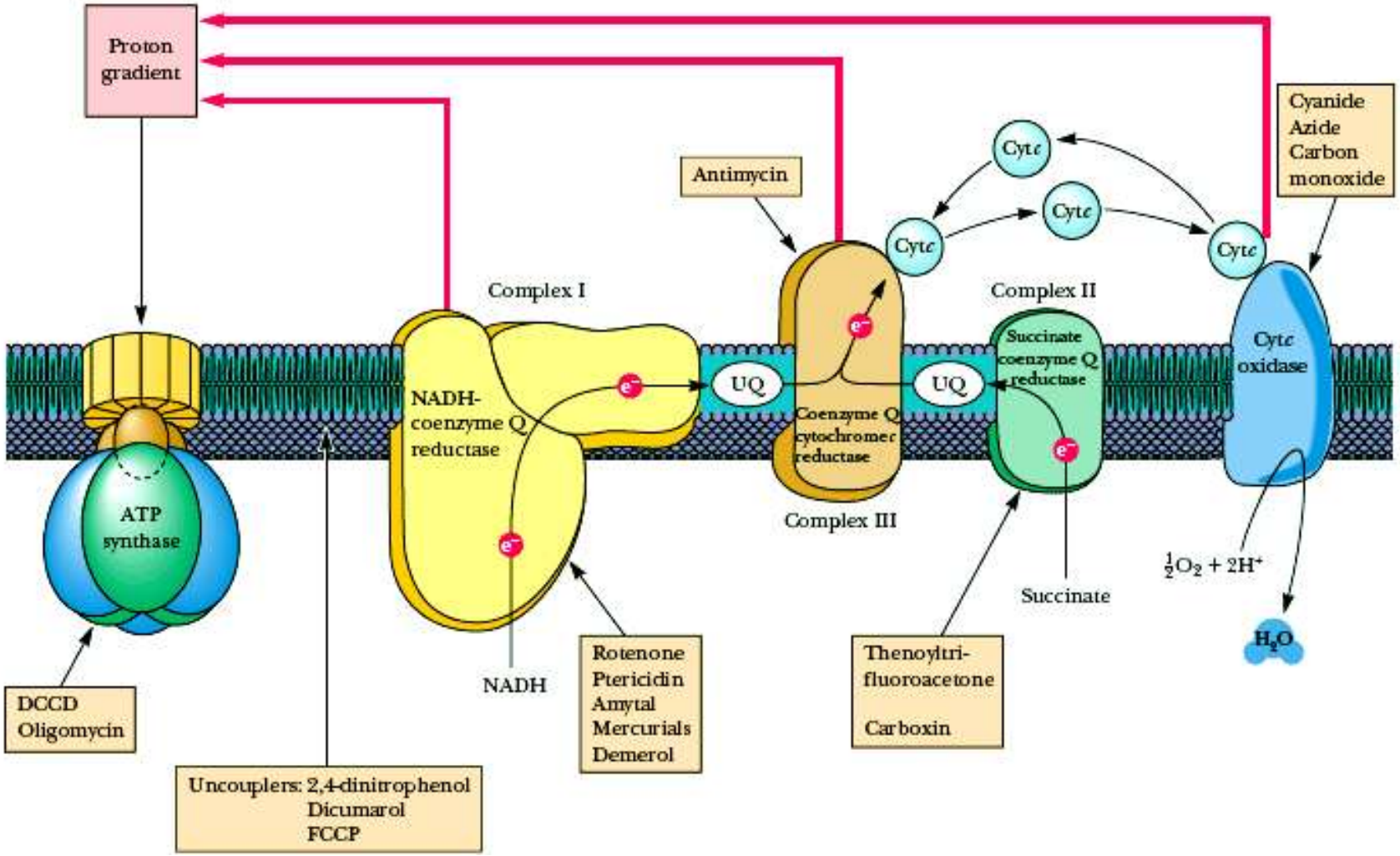


- There are 3 cycles in ETC functioning: F-cycle, Q-cycle and O-cycle.
- Proton pumping result in electrochemical gradient $\Delta\mu H^+$ formation.
- Finally it is used for ATP formation.

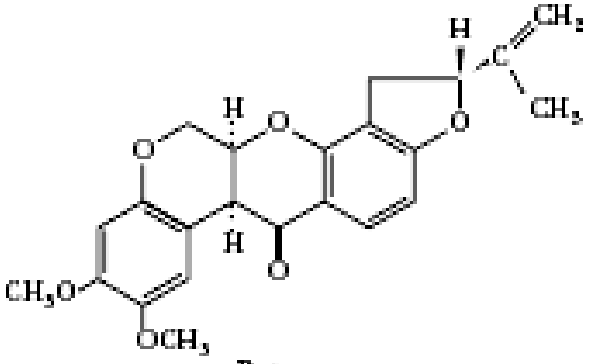
Inhibitors of Oxidative Phosphorylation

Name	Function	Site of Action
Rotenone	e^- transport inhibitor	Complex I
Amytal	e^- transport inhibitor	Complex I
Antimycin A	e^- transport inhibitor	Complex III
Cyanide	e^- transport inhibitor	Complex IV
Carbon Monoxide	e^- transport inhibitor	Complex IV
Azide	e^- transport inhibitor	Complex IV
2,4,-dinitrophenol	Uncoupling agent	transmembrane H^+ carrier
Pentachlorophenol	Uncoupling agent	transmembrane H^+ carrier
Oligomycin	Inhibits **ATP synthase**	OSCP fraction of **ATP synthase**

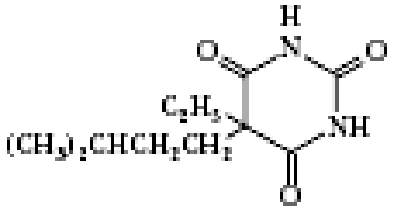
The Sites of Action of Several Inhibitors of ETC and/or OP



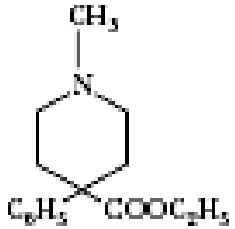
The Structures of Several Inhibitors of ETC and OP



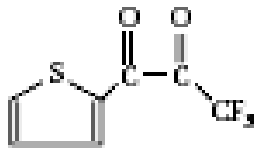
Rotenone



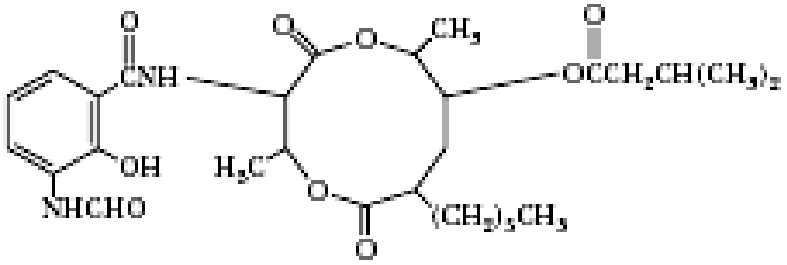
Amytal
(amobarbital)



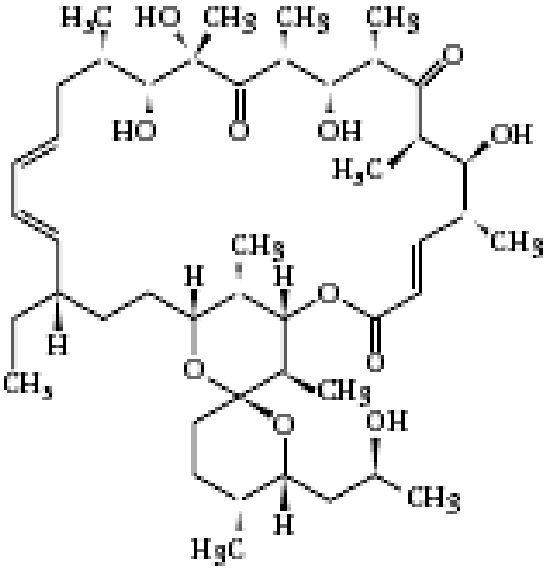
Demerol
(meperdine)



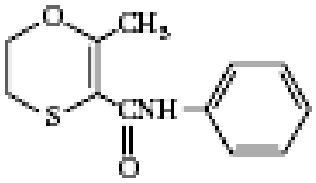
2-Thenoyltrifluoroacetone



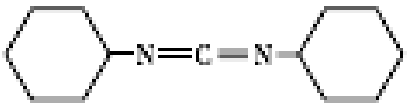
Antimycin A_1



Oligomycin A

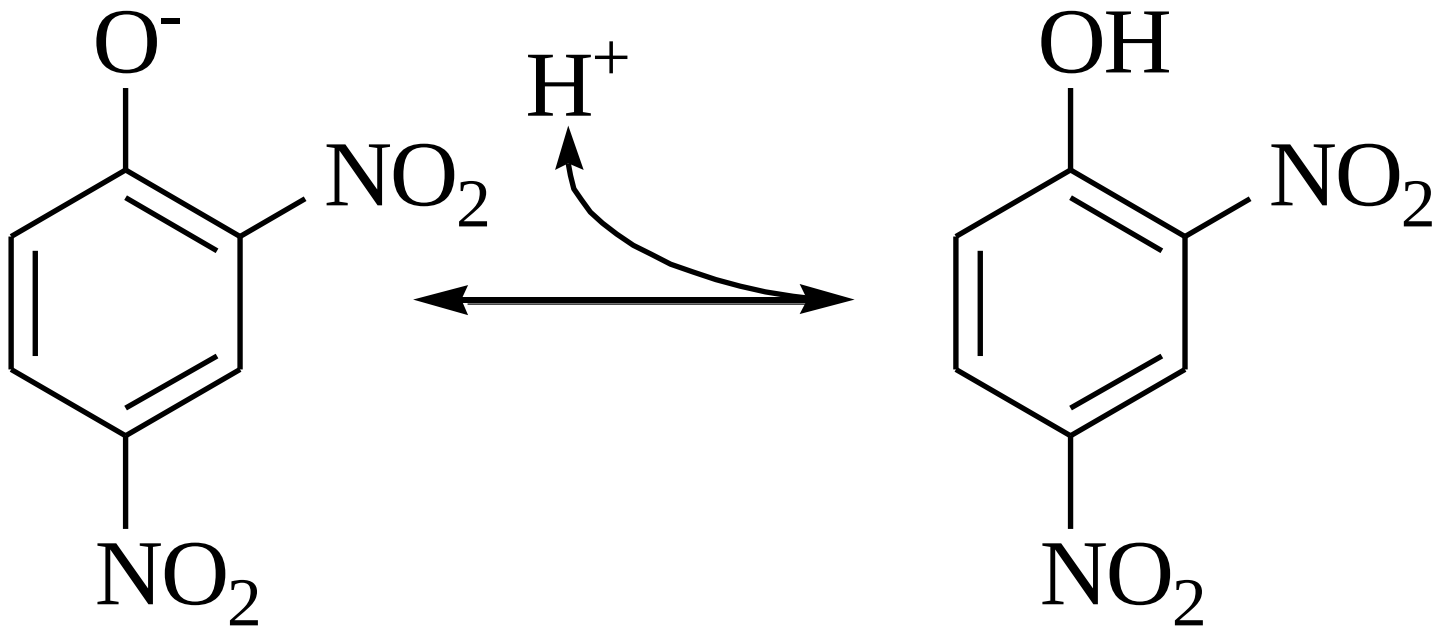


Carboxin



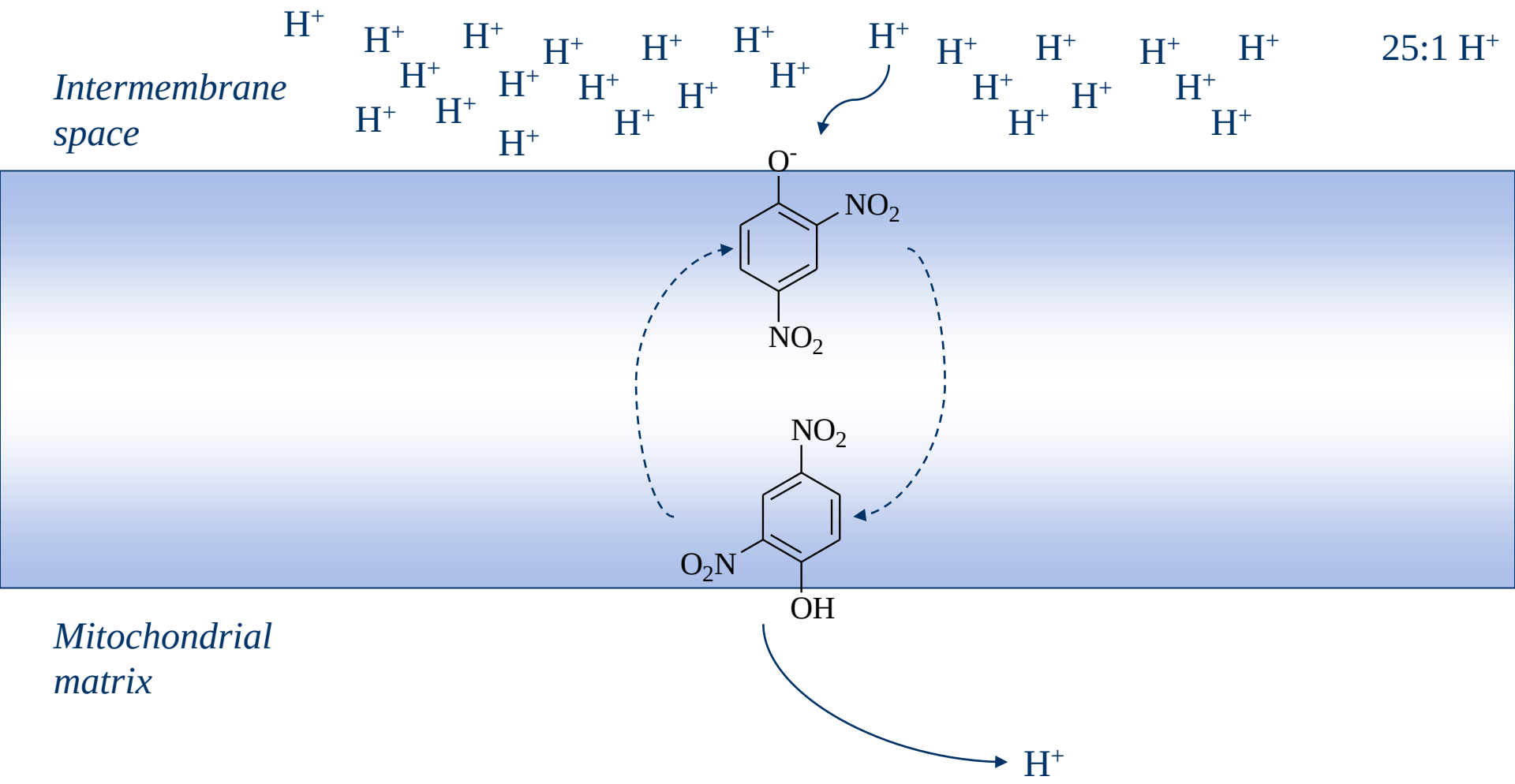
Dicyclohexylcarbodiimide (DCCD)

Uncoupler Action



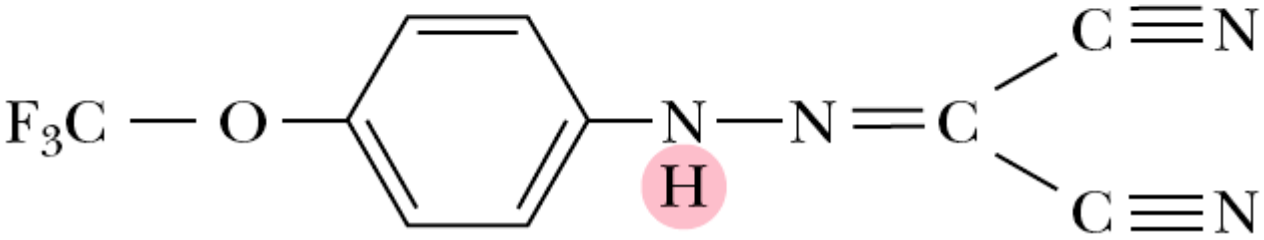
- 2,4-Dinitrophenol (2,4-DNP) can uptake proton from the intermembrane space and transports it back to the mitochondrial matrix.

Uncouplers: 2,4-DNP decreases ΔμH+

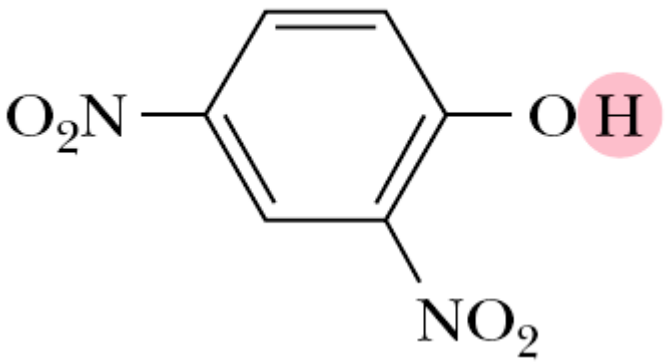


Several Uncouplers of OP

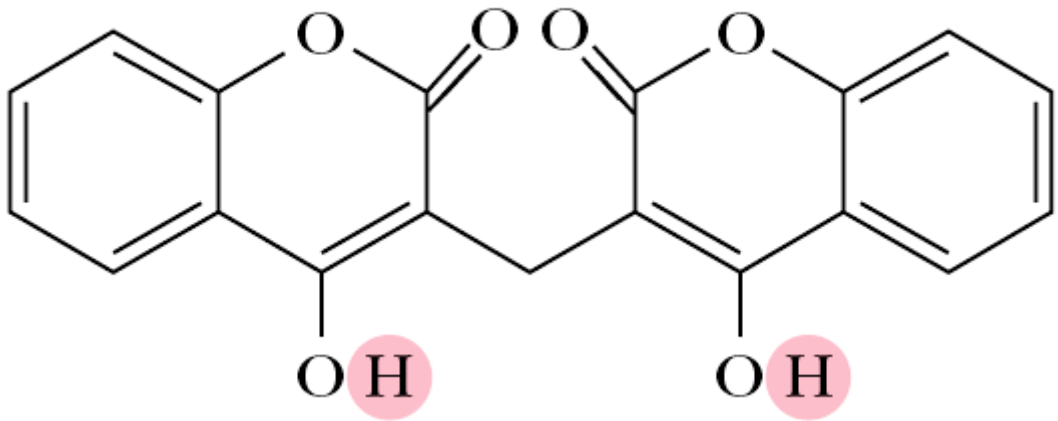
Carbonyl cyanide-p-trifluoro-methoxyphenyl hydrazone
—best known as **FCCP**; for **F**luoro **C**arbonyl **C**yanide **P**henylhydrazone



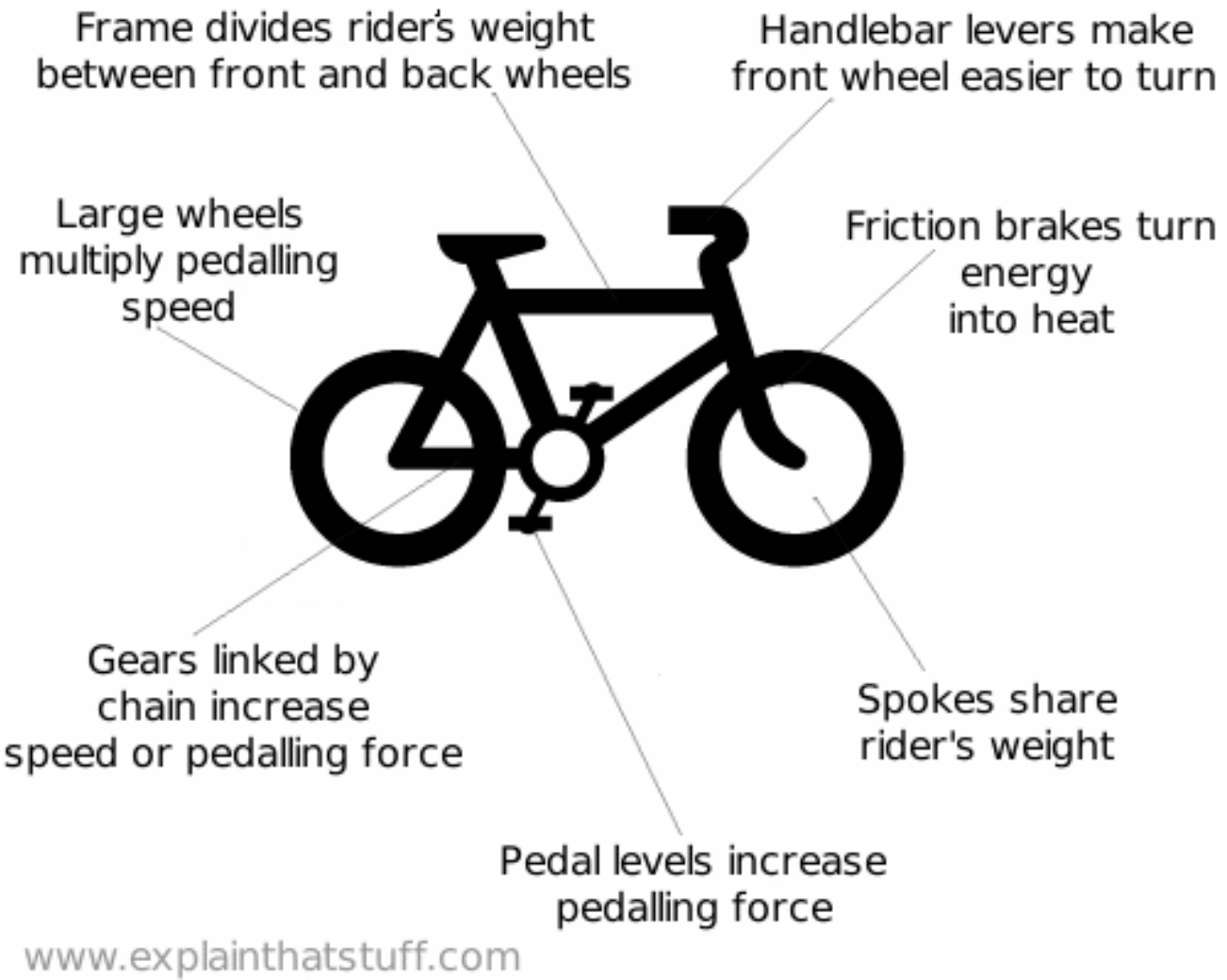
Dinitrophenol



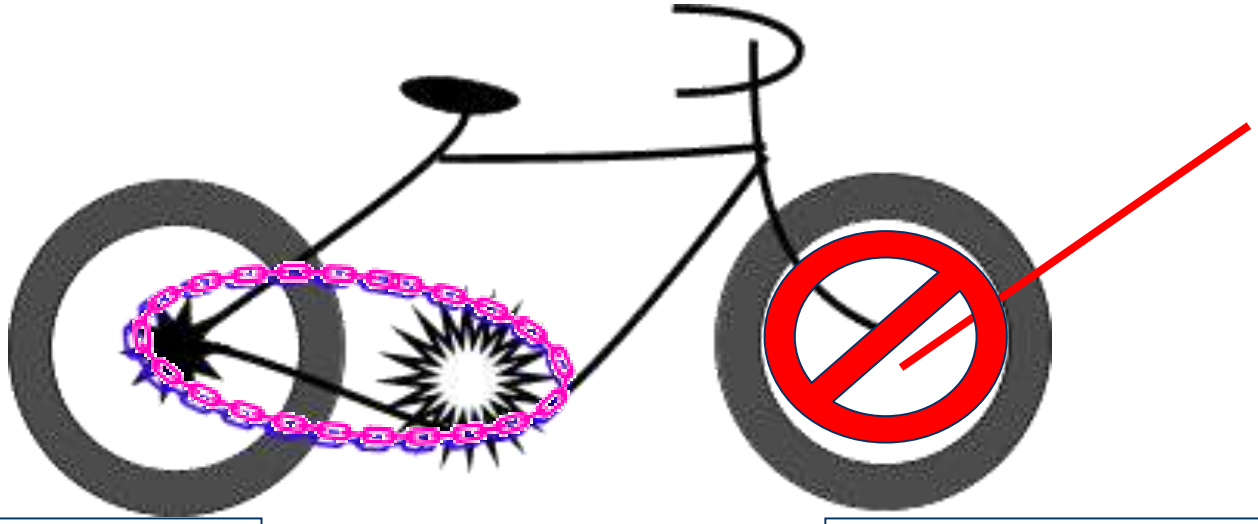
Dicumarol



Some words about bicycle…



Inhibitors vs Uncouplers: the Model



Uncouplers **loosen** the chain.
Pedal rotation is possible.
Wheel rotation decreases.

Inhibitors **block** the wheel.
Pedal and wheel rotation are impossible.

Endogenous Uncouplers Enable Organisms To Generate Heat

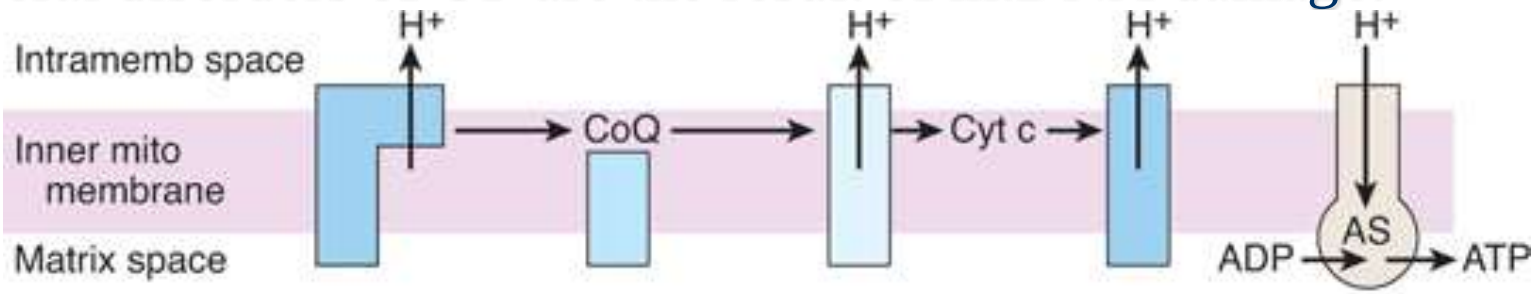
- Certain cold-adapted animals, hibernating animals, and newborn animals generate large amounts of heat by uncoupling oxidative phosphorylation.
  - Adipose tissue in these organisms contains so many mitochondria that it is called *brown adipose tissue* for the color imparted by the mitochondria.
  - The inner membrane of brown adipose tissue mitochondria contains an endogenous protein called **thermogenin** (literally, "heat maker"), or *uncoupling protein*, that creates a passive proton channel through which protons flow from the cytosol to the matrix.

P/O Ratio

- Electrons that enter the chain from NADH supports the synthesis of ≈3 moles of ATP.
- Electrons that enter the chain from $FADH_2$ supports the synthesis of ≈2 moles of ATP.
- The P/O ratio refers to the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed.
  - NADH P/O = 3
  - $FADH_2$ P/O = 2
  - Ascorbate P/O = 1

Disorders of Mitochondrial Oxidative Phosphorylation

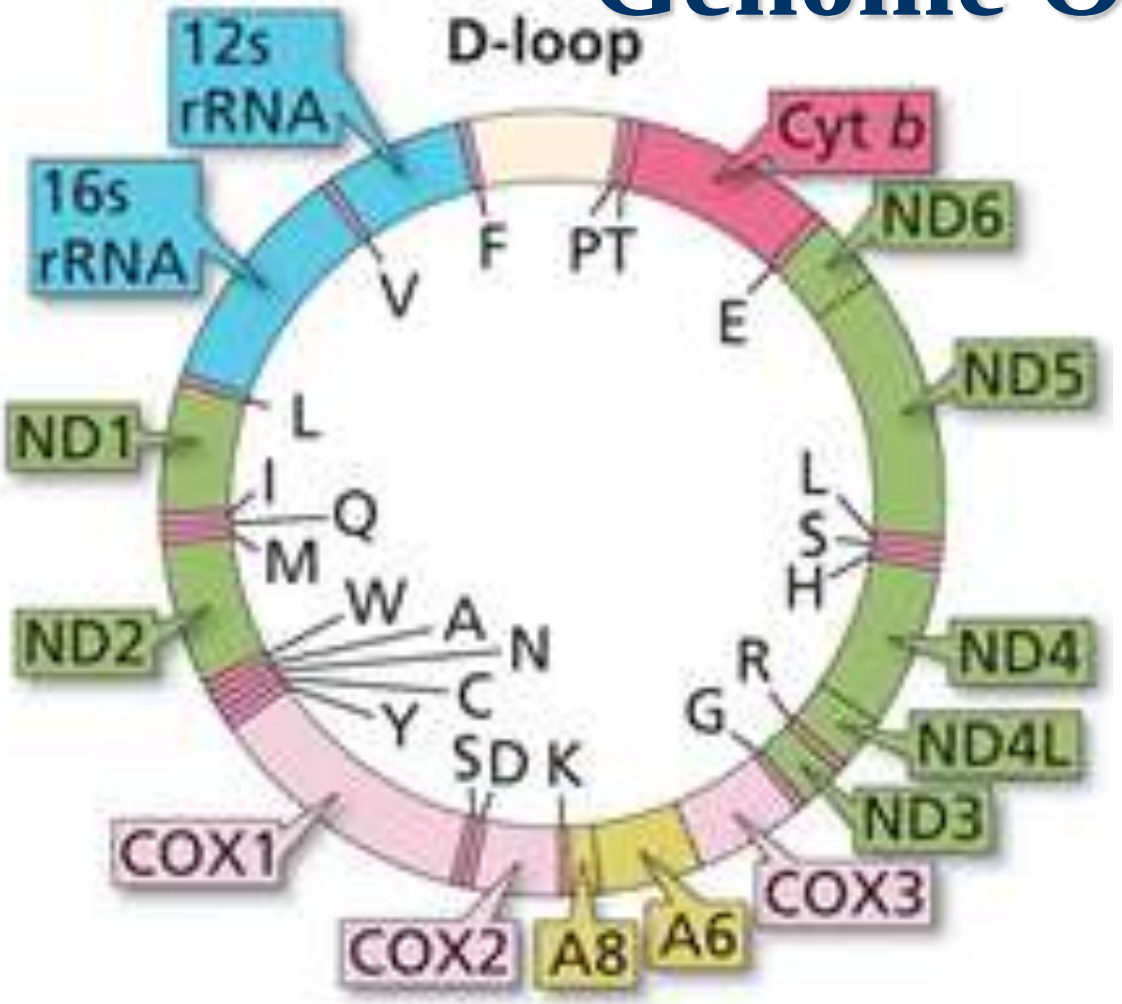
- Mitochondria contain DNA (mtDNA).
- Some components of ETC are coded in mtDNA. Others – in nuclear DNA.
- Several disorders of OP are the result of mtDNA damage.



Complex	I	II	III	IV	V
Subunits from mDNA	7	0	1	3	2
Subunits from nDNA	39	4	10	10	14

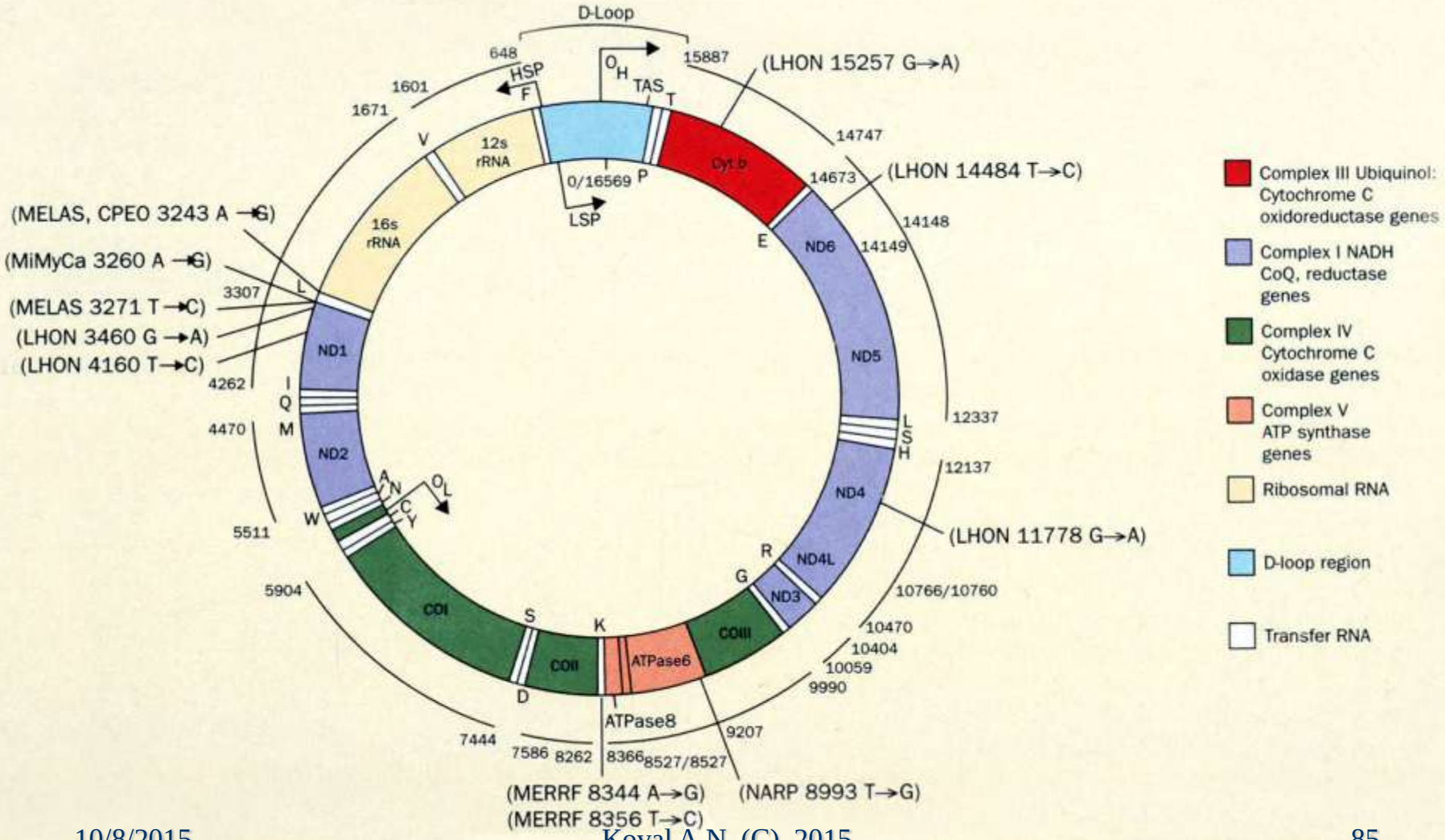
Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Mitochondrial Genome Organization



Mitochondrial genome. Mitochondrial genomes are small, circular, double-stranded DNA molecules. They contain genes for ribosomal RNAs (12S rRNA, 16S rRNA) and tRNAs (labeled according to the amino acid they carry). The human mitochondrial genome, shown here, is only 16,589 bp in size and it encodes only a few of the subunits of the electron transport complexes. Genes for the subunits of complex I are colored green, a complex III subunit is purple, complex IV subunits are pink, and complex V subunits are yellow. The D-loop is a highly variable region required for DNA replication. Sequences of individual D-loop regions have been used to trace the evolution of modern humans providing early evidence that we all descend from a population in Africa.

MitoDNA Mutations



Clinical Manifestation and Treatment of Mito Disorders

- Manifestation
  - Muscle cramping and weakness,
  - Fatigue,
  - Lactic acidosis,
  - CNS dysfunction,
  - Vision problems.
- Treatment
  - Is difficult and often unsuccesfull
  - In some cases can be helpful ubiquinone, vitamin C, menadione.

Some Mitochondrial Diseases

- The names of mitochondrial diseases are often complex and usually are described by abbreviations.
  - **LHON**, Lebers hereditary optical neuropathy;
  - **MERRF**, myoclonic epilepsy and ragged-red-fiber disease;
  - **MELAS**, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes;
  - **NARP**, neurological muscle weakness,ataxia, and retinitis pigmentosa;
  - **Leigh disease.**
  - **SNE**, subacute necrotizing encephalomyelopathy;
  - **KSS**, Kearns–Sayre syndrome;
  - **CPEO**, chronic progressive external ophthalmoplegia.
- **LHON** is a hereditary disease that often leads to sudden blindness from death of the optic nerve especially among males. Any one of several point mutations in subunits ND1, 2, 4, 5, and 6 of NADH dehydrogenase.

Can Mitochondrial Diseases be Treated?

- Attempts are being made to improve the function of impaired mitochondria by adding large amounts of ubiquinone, vitamin K, thiamin, riboflavin, and succinate to the diet. One report suggests that mitochondrial decay during aging can be reversed by administration of *N*-acetylcarnitine.

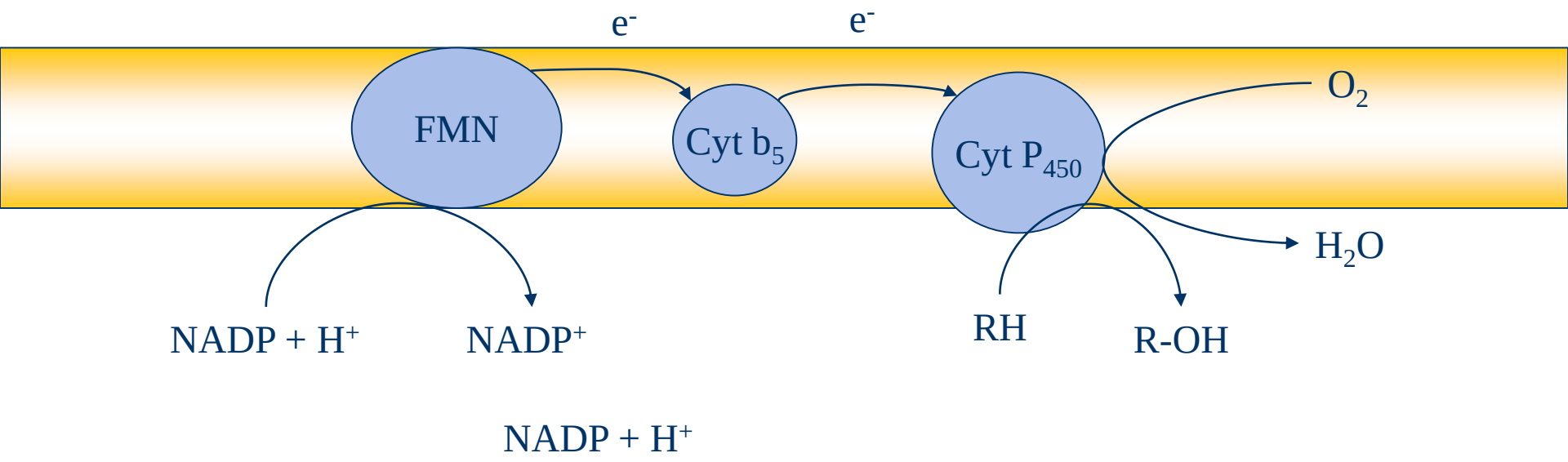
Cytochromes P_{450} are monooxygenases important for the detoxification of many drugs & for the hydroxylation of steroids

- Cytochromes P_{450} - superfamily of heme-containing monooxgenases,
  - > than 1000 such enzymes are known.
- Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes, which in turn are oxidized by substrates in a series of enzymatic reactions collectively known as the **hydroxylase cycle**.
- In liver microsomes, cytochromes P_{450} are found together with **cytochrome *b*5** and have an important role in detoxification.
  - Benzpyrene, aminopyrine, aniline, morphine, and benzphetamine are hydroxylated, increasing their solubility and aiding their excretion.
  - Many drugs such as phenobarbital have the ability to induce the formation of microsomal enzymes and of cytochromes P450.

Monooxygenase System (Microsomal Oxidation)

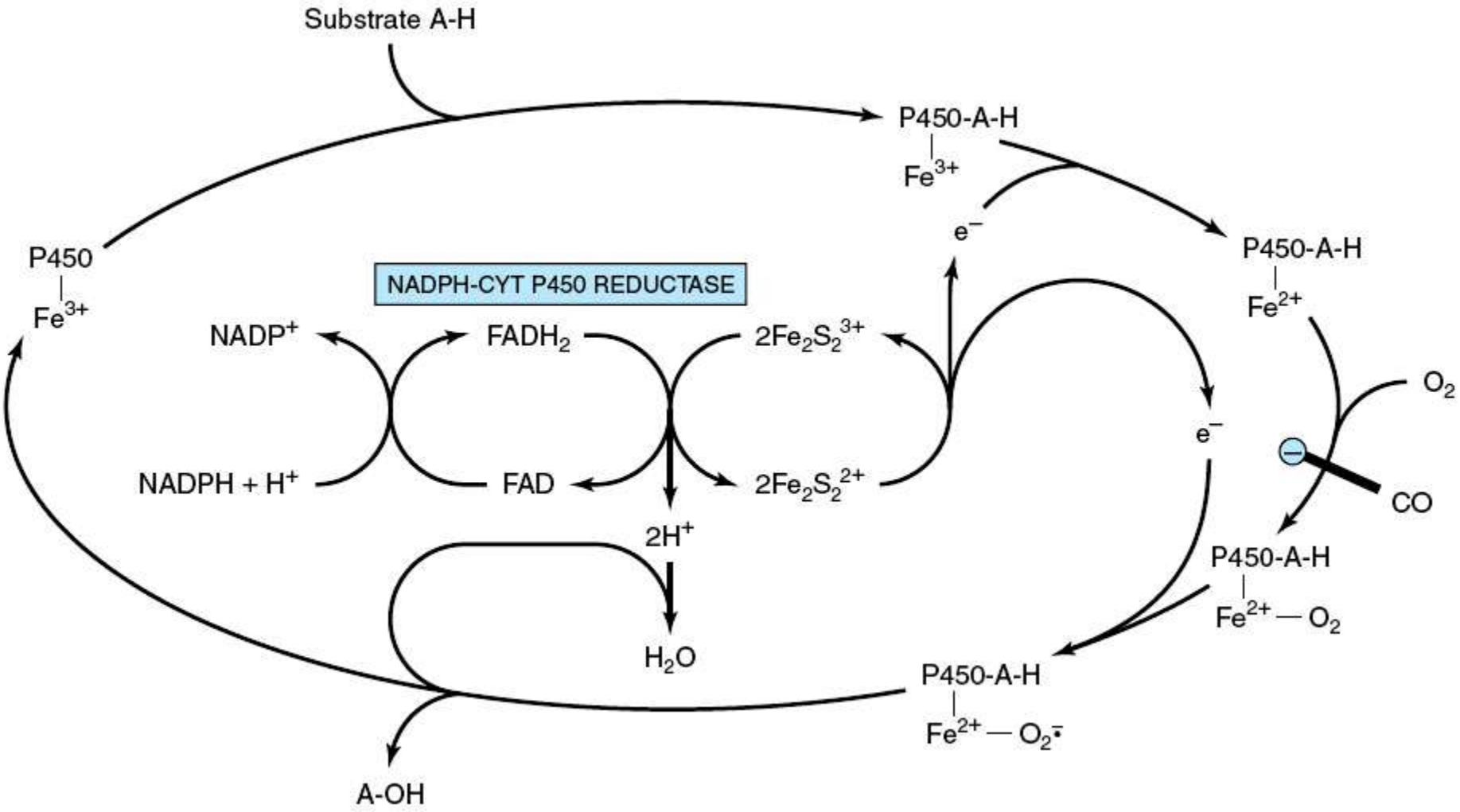
- The substrate can be oxidized by incorporation of one atom of O_2.
- The enzymes are **monooxygenases** or **cytochrome P_{450} -** also **mixed function oxidase**.
- The enzymes are associated with the smooth endoplasmic reticulum, preparated as *microsomes*.
- $RH + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$

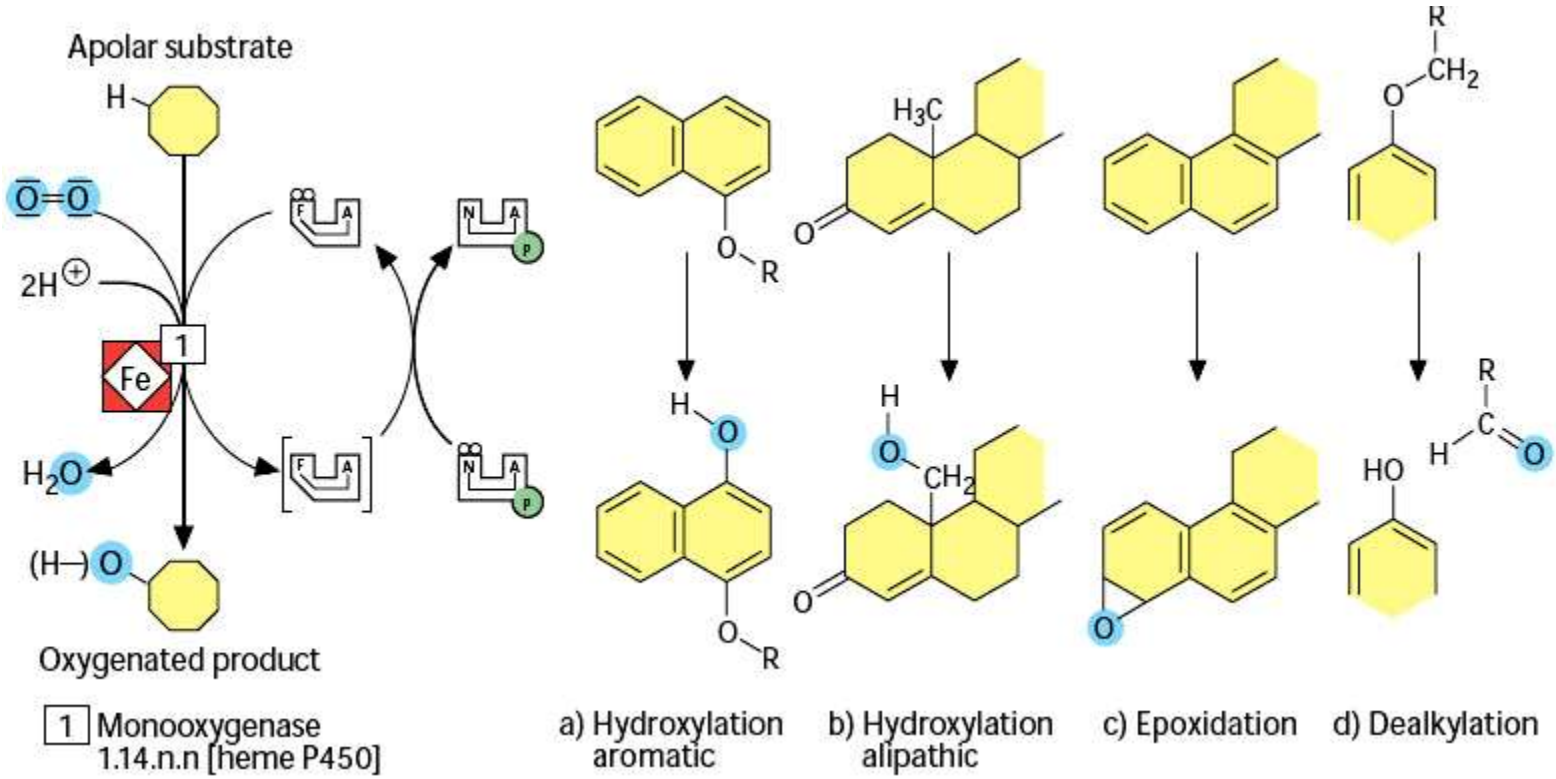
Functioning of Microsomal Respiratory Chain

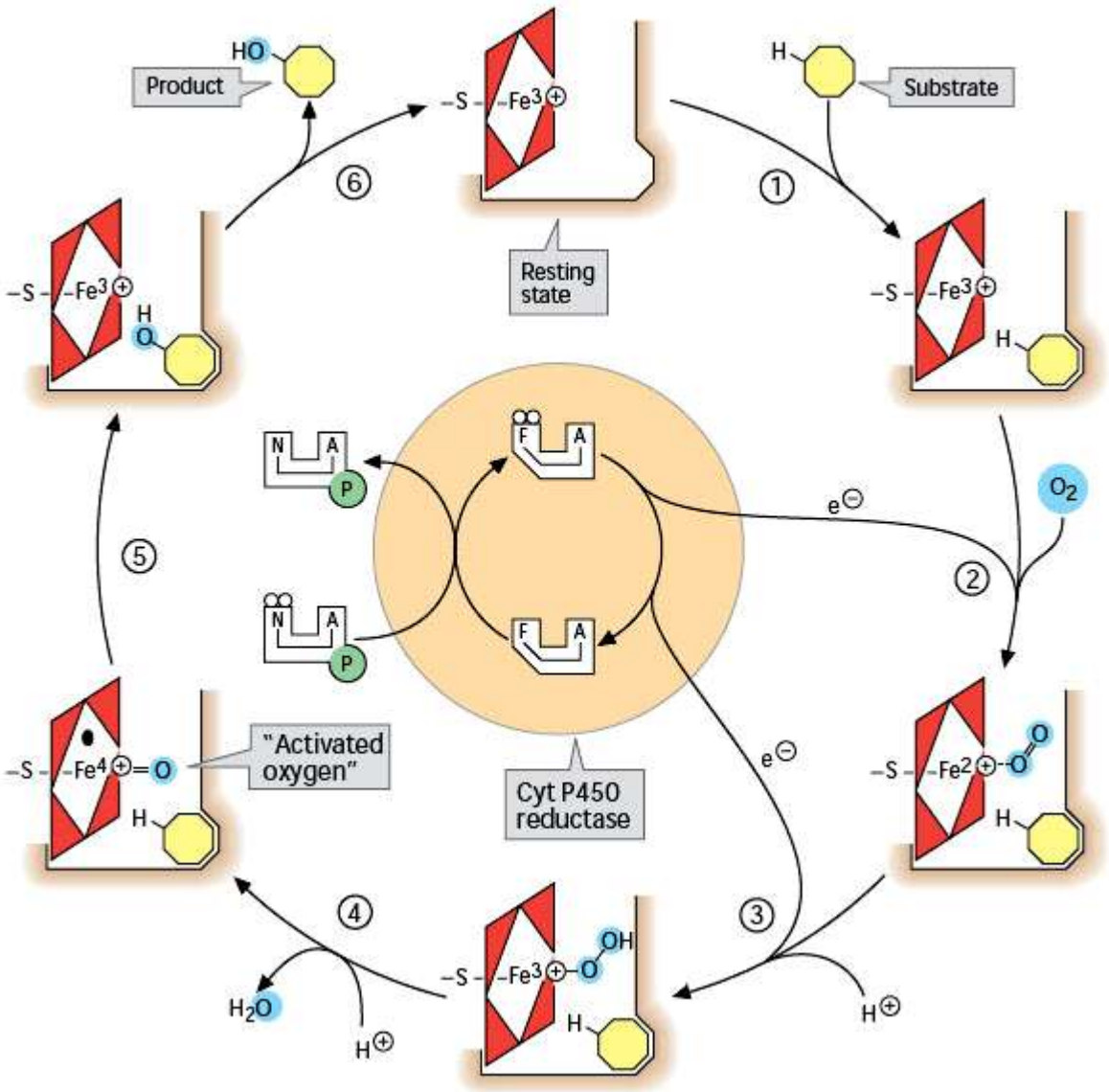


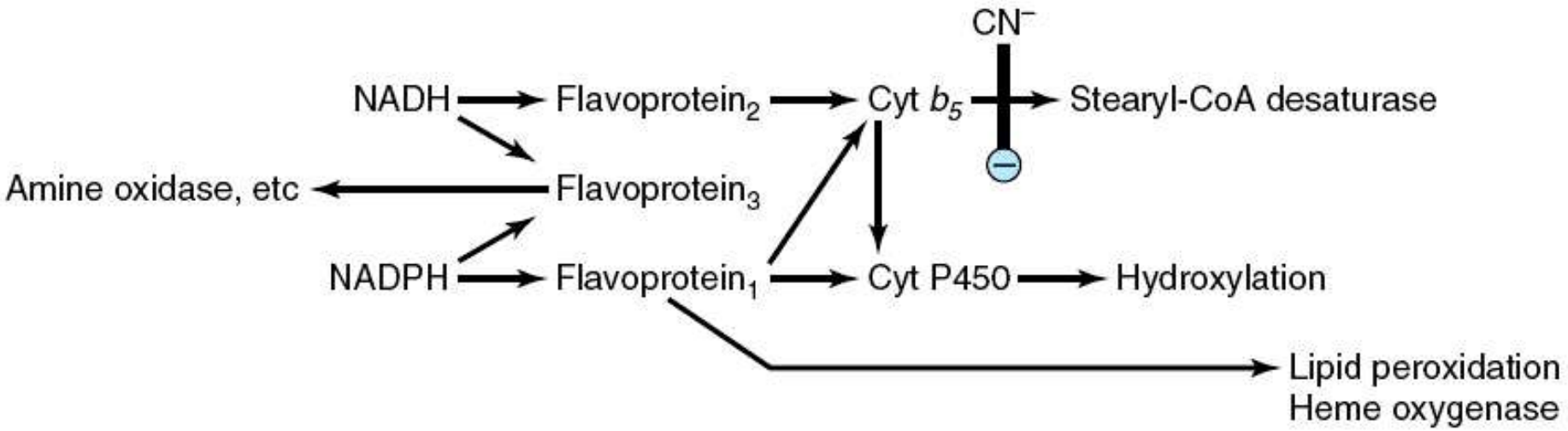
NADP + H+

The Mechanism of P450-reaction




Apolar substrate
H
O=O
2H⊕
1
Fe
H2O
(H–) O
Oxygenated product
1 Monooxygenase
1.14.n.n [heme P450]
F
A
N
A
P
H3C
O
R
CH2
HO
a) Hydroxylation aromatic
b) Hydroxylation alipathic
c) Epoxidation
d) Dealkylation

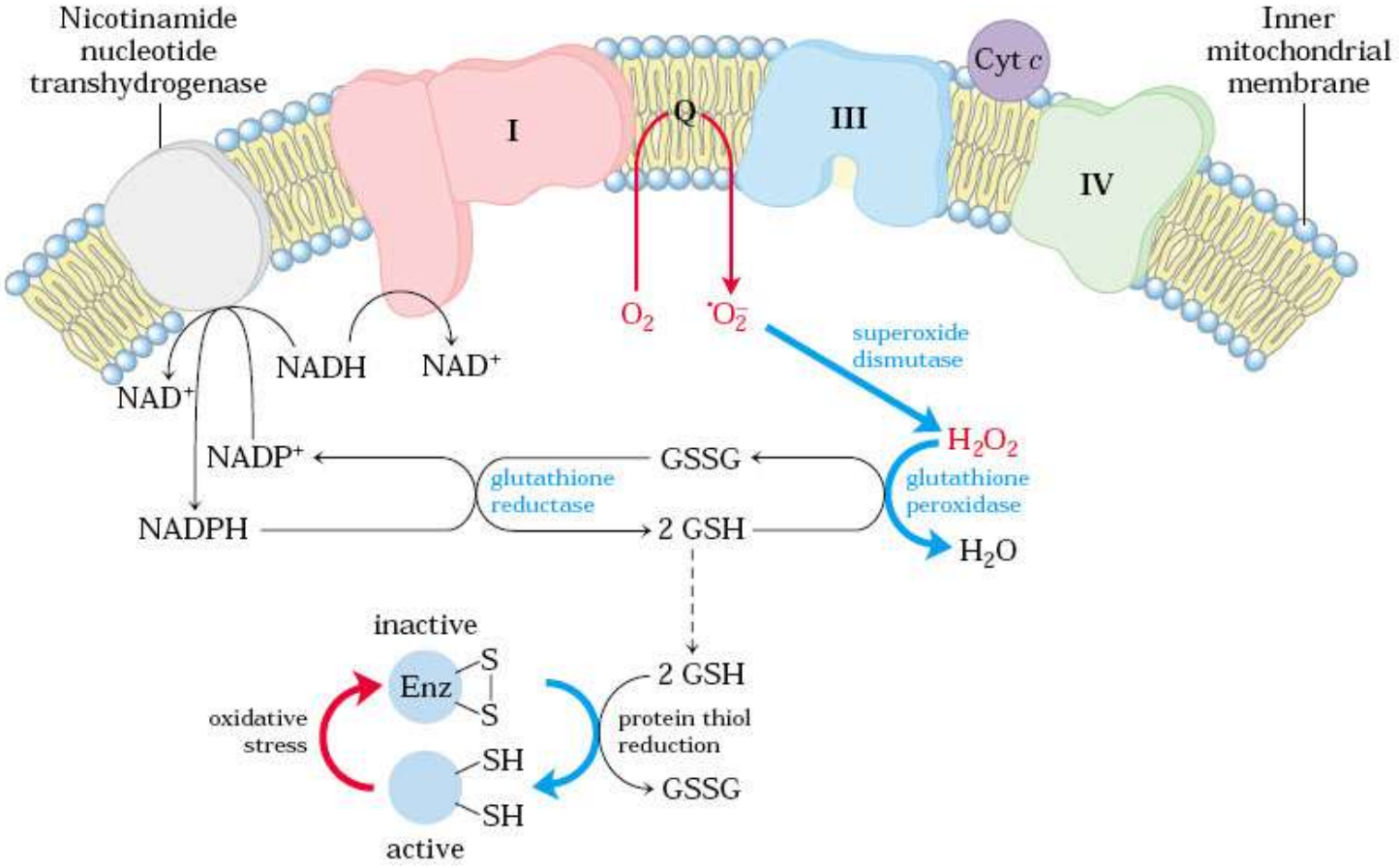

Product
HO
Substrate
H
Resting state
–S
–Fe3
Fe3
H
O
1
2
3
4
5
6
N
A
P
F
A
e⊖
O2
"Activated oxygen"
–Fe4
=O
Cyt P450 reductase
–Fe2
O
–Fe3
OH
H2O
H⊕


CN⁻
NADH
Flavoprotein2
Cyt b5
Stearyl-CoA desaturase
Amine oxidase, etc
Flavoprotein3
NADPH
Flavoprotein1
Cyt P450
Hydroxylation
Lipid peroxidation
Heme oxygenase

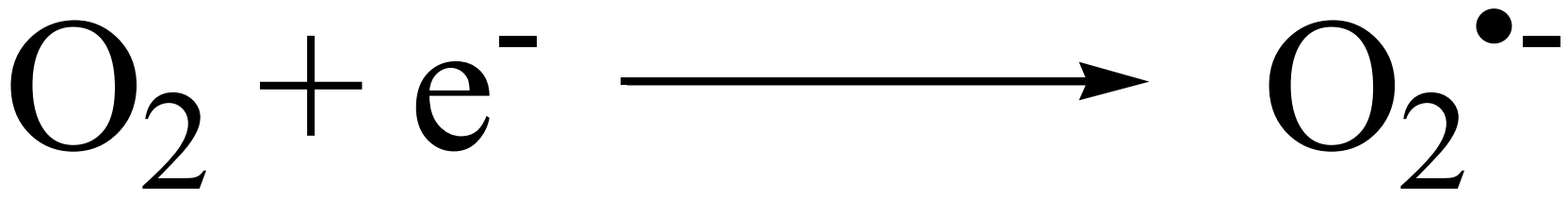
Peroxidation Processes

- There are some conditions when reactive oxygen species (ROS) may be formed in organism:
  - E.g., hypoxia, high calory diet, alcohol intake, mitochondrial ETC damage (mito diseases or ageing), ionizing radiation.
- During long years of evolution our mitochondria elaborated the mechanism of defense against such hypoxic conditions.
  - They produce ROS as a signal of hypoxia.

Mitochondrial Production of Superoxide-anion

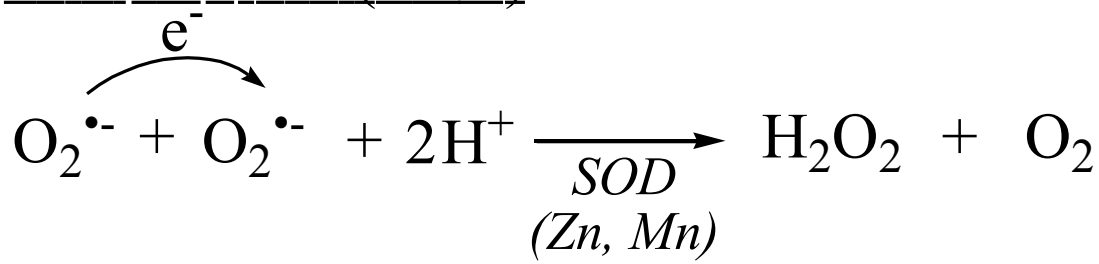


Formation of Superoxide-anion Radical

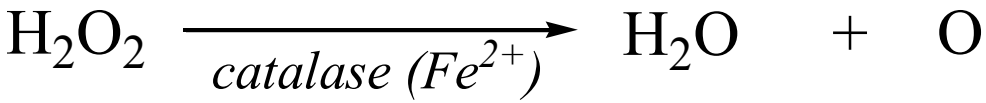


Antioxidant Defense Enzymes

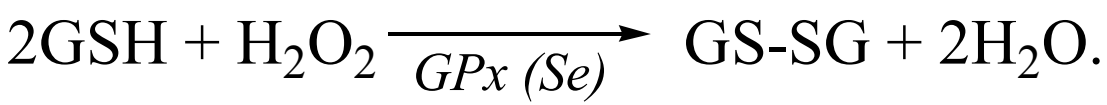
Superoxide dismutase (SOD)



Catalase

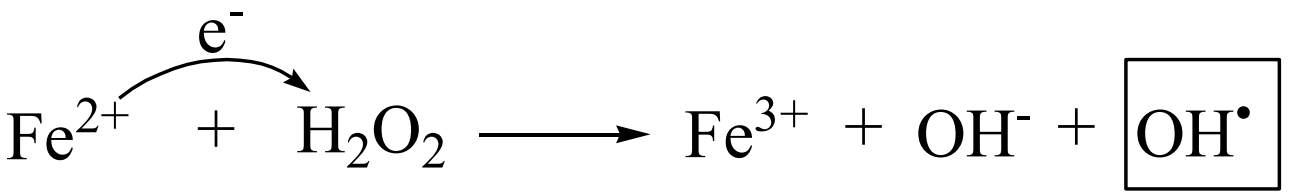


Glutathione peroxidase (GPx)



Hydroxyl-radical formation

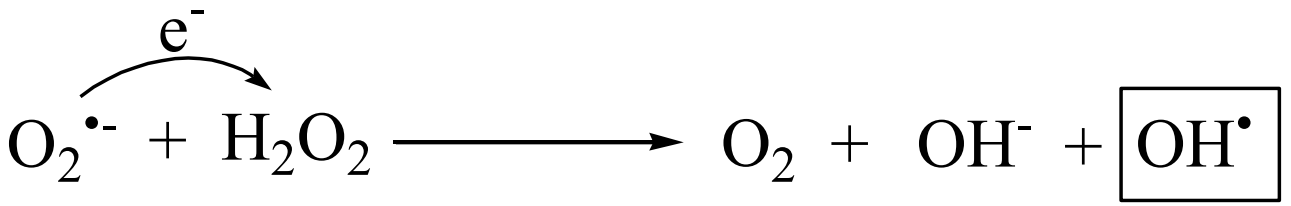
Fenton reaction



hydroxyl-radical

$t_{1/2} = 10^{-9} s$

Haber-Weiss reaction




Thank you
for your attention